Talbot, H., McClymont, E., Inglis, G. N., Evershed, R. P., & Pancost, R. D. (2016). Origin and preservation of bacteriohopanepolyol signatures in *Sphagnum* peat from Bissendorfer Moor (Germany). *Organic Geochemistry*, *97*, 95-110. https://doi.org/10.1016/j.orggeochem.2016.04.011

# Origin and preservation of bacteriohopanepolyolsignatures in *Sphagnum* peat from Bissendorfer Moor (Germany)

Helen M. Talbot[a,*], Erin L. McClymont[b], Gordon N. Inglis[c,d], Richard P. Evershed[c], Richard D. Pancost[c,d]

[a]*School of Civil Engineering and Geoscience, Drummond Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK.*

[b]*Department of Geography, University of Durham, Durham, UK.*

[c]*Organic Geochemistry Unit (OGU), Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK.*

[d]*Cabot Institute, University of Bristol, Bristol BS8 1UJ, UK*

## Abstract

Distributions of bacteriohopanepolyols (BHPs) were investigated in a peat core from the Bissendorfer Moor (Germany) in order to test the utility of BHPs as indicators of microbial processes in peats. Between 13 and 22 BHPs were identified in each sample (23 structures in total), with total concentrations ranging from 160 – 2800 μg $g^{-1}_{TOC}$. We have tentatively ascribed sources of most BHPs observed at this site via comparison of known BHPs source organisms with recent microbiological studies on the peat microbiome. Members of the Alpha-, Beta- and Gammaproteobacteria and specifically the genera *Burkholderia*, *Bradyrhizobium* and *Rhodoblastus*, as well as other phyla including the cyanobacteria, Acidobacteria and Acetobacteria are amongst the most likely sources. Additionally, BHP signatures which could be assigned directly to methane oxidising bacteria (35-aminobacteriohopanepentol and 35-aminobacteriohopanepentol) were

present only at very low levels, supporting previous studies which have shown that the majority of precursor organisms biosynthesising hopanoids in peat environments are heterotrophs. The surface layers also contained a highly unusual signature comprising high concentrations of unsaturated compounds, including unsaturated bacteriohopanetetrol pseudopentose, which has previously only been reported in *Gloeocapsa* cyanobacteria. This genus is known to occur in symbiotic association with host *Sphagnum* species, and has the ability to fix atmospheric nitrogen which is a well known trait amongst members of the peat microbiome and amongst hopanoid producing microorganisms. The apparent capacity for hopanoids to protect organisms from external stresses such as low pH is therefore likely to be a significant factor accounting for the high BHP contributions from heterotrophs, methanotrophs and phototrophic organisms in *Sphagnum* peats.

* Corresponding author. 0191 208 6426

*E-mail address*: helen.talbot@ncl.ac.uk (Helen Talbot)

## 1. Introduction

Peatlands contain vast stocks of organic carbon (OC) with Northern areas (including boreal and subarctic peatlands above 45° N) currently storing around 547 Gt OC as waterlogged peat (Yu et al., 2010). Carbon cycling in peats is important in terms of degradation or preservation of organic matter, with the former culminating in methanogenesis (Ciais et al., 2013). These processes are affected by changes in temperature, water table depth and organic matter content (Gorham, 1991; Kotsyurbenko et al., 2007 and references therein). New proxies for unravelling these processes in modern and ancient settings could therefore be useful for elucidating the environmental controls regulating carbon cycling and methane emissions (e.g. Pancost et al., 2011; Chambers et al., 2012; Zheng et al., 2014).

Until recently, microbiological investigations of organisms inhabiting peat bogs, including their adaptation to harsh conditions of low pH and low nutrient input, has largely focussed on organisms with specific metabolisms such as methanogenic archaea and methanotrophic bacteria as they have direct relevance to carbon cycling and climate change (see review by Andersen et al., 2013 and references therein). However, the use of visualisation techniques such as fluorescence in situ hibridisation and confocal laser scanning microscopy have revealed that *Sphagnum* mosses host a wide range of endophytic organisms in their dead hyaline cells (e.g. Opelt and Berg, 2004; Opelt et al., 2007; Bragina et al., 2012a; Shcherbakov et al., 2013). Of the organisms inhabiting the hyaline cells, methanotrophs are of particular interest as they limit the release of methane to the atmosphere and have also been shown to provide $CO_2$ to the host *Sphagnum* plants (Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010). They may also provide a significant source of fixed nitrogen which is otherwise typically limited in these systems under low atmospheric N deposition (Auman et al., 2001; Larmola et al., 2014). Studies of genes encoding nitrogenase reductase proteins (*nifH*) in *Sphagnum* mosses have

revealed that they are mainly derived from *Alphaproteobacteria*, a group of highly diverse organisms including phototrophs, heterotrophs and methanotrophs (Bragina et al., 2012b). However, the ability to fix $N_2$ is also widely distributed in other phyla across the *Sphagnum* peat microbiome; for example Betaproteobacteria of the genus *Burkholderia* sp., are also known to be important sources of fixed $N_2$ in peatlands (e.g. Belova et al., 2006; Opelt et al., 2007; Bragina et al., 2013; Shcherbakov et al., 2013).

The ability to fix nitrogen and/or oxidise methane is a very common, although not universal trait, amongst organisms which produce hopanoids (e.g. Pearson et al., 2007; Blumenberg et al., 2012; Ricci et al., 2014). Hopanoids are pentacyclic triterpenoid lipids produced by some bacteria (e.g. Rohmer et al., 1984; Ourisson et al., 1987; Ourisson and Rohmer, 1992; Farrimond et al., 1998; Talbot et al., 2008; Pearson et al., 2009). These compounds typically consist of a $C_{30}$ ring system (**I**; see Appendix), although variations such as methylation at C-2 (**II**) or C-3 (**III**) and unsaturation at C-6 and/or C-11 (**IV**-**VI**) do occur; however, the latter are rarely observed in environmental materials. They can also contain an extended polyfunctionalised side chain derived from D-ribose (Flesch and Rohmer, 1988) and are termed bacteriohopanepolyols (BHPs).

Typical structures have functional groups present at C-32, 33, 34 and 35, the most common example being bacteriohopane-32,33,34,35-tetrol (BHT, **Ia**). Structures with additional hydroxyl moieties at C-31 and/or C-30 are also known (e.g. Rohmer, 1993). BHPs can be broadly assigned into one of two categories “non-composite” and “composite” with the former only containing a simple functionality at the C-35 position such as -OH or $–NH_2$ including BHT (**Ia**) and 35-aminobacteriohopane-32,33,34-triol (**Id**) whilst the latter have a more complex functionality such as a sugar or aminosugar at C-35. Some structures are only known to be produced by certain groups of organisms (see summary in Table 1; Rohmer, 1993; Talbot and Farrimond, 2007; Talbot et al., 2008, 2014) providing a potentially powerful tool for profiling the microbial community, as recently demonstrated in

Siberian permafrost by comparison with genomic profiling (Höfle et al., 2015). Other BHPs have been related to specific environmental settings. For example adenosylhopane (**Ig**) is a biosynthetic intermediate in the synthesis of other elongated hopanoids (Bradley et al., 2010), which only seems to accumulate significantly in soils (e.g. Cooke et al., 2008a; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011). These compounds have been used to trace the transport of terrestrial (soil) organic matter to marine sediments (e.g. Cooke et al., 2008b; Cooke et al., 2009; Handley et al., 2010; Taylor and Harvey, 2011; Zhu et al., 2011; Doğrul Selver et al., 2012, 2015; Wagner et al., 2014).

BHPs appear to perform a regulating and rigidifying function similar to that of some sterols in eukaryotes (Kannenberg and Poralla, 1999 and references therein; Sáenz et al., 2012). Although their exact function remains unclear, their regulation has been linked to a variety of environmental factors including temperature, pH, moisture limitation (e.g. Kannenberg and Poralla, 1999; Poralla et al., 2000; Joyeux et al., 2004; Welander et al. 2009; Kulkarni et al., 2013) and also growth phase (Joyeux et al., 2004; Doughty et al., 2009; Welander and Summons, 2012).

Classically, investigations of bacterial communities in peat bogs using hopanoids have focussed on the geohopanoid degradation products of BHPs, such as hopanoic acids, hopanols and hopanes (e.g. Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995; Pancost et al., 2003; McClymont et al., 2008) and total hopanoid abundance has been used to study relative changes in bacterial biomass in the past (Pancost et al., 2003). In comparison, there have been few previous reports of intact polyfunctionalised BHPs in peat. BHT was first reported by Ries-Kautt and Albrecht (1989). More recently Kim et al. (2011) found between 5 and 16 BHPs in four peat samples from the catchment of the River Têt (France). Van Winden et al. (2012a) also reported the BHP composition of *Sphagnum* moss and underlying peat (to a depth of 10 cm) from a site at Moorhouse (UK), an acidic ombrotrophic blanket bog, and identified up to 13 BHP structures, including

markers for methane oxidising bacteria, albeit in low abundance. Similar distributions were also reported from a *Sphagnum* peat core from Belgium between 13 and 100 cm depth (van Winden et al., 2012b).

These earlier studies indicate the potential for BHPs to be well preserved in peat but have been limited in age/depth resolution, typically less than 400 years in age and likely within the zone of a thriving bacterial population or focused on intermediate depths without comparable surface samples (van Winden et al., 2012b). The persistence of these signatures to greater depth in peat deposits has not been investigated. Therefore, we report for the first time a full characterisation of intact BHPs in peat samples from Bissendorfer Moor (BM, Germany) to a depth of 410 cm and an age of ~2,900 cal. yr BP (Pancost et al., 2011). We focus in particular on the surface samples within the range of seasonal water table fluctuations. The primary objectives were to explore whether the diversity of BHPs reflects the predominantly heterotrophic microbially-mediated processes that occur in peat, determine whether nitrogen fixation and methane oxidation signatures could be identified against this background, and to explore how well such BHP-based signals are preserved at depth. A secondary objective was to compare peat BHP distributions to those determined for other settings; it is expected that they will be broadly similar to soil distributions, but specific characteristics of the peat environment, including nutrient limitation, a relatively low pH and strongly reducing conditions, could induce profound differences.

## 2. Materials and methods

### *2.1. Site description and samples*

Peat samples were collected from Bissendorfer Moor, Germany (9.683065 E, 52.506028 N; McClymont et al., 2011). This ombrotrophic bog lies 50 metres above sea-level and has been designated as a nature conservation area of 498 ha since 1971. The

vegetation comprises a treeless central area with mainly cotton grass and heather. Hollows and hummocks are not sharply differentiated with *Sphagnum magellanicum*, *S. rubellum*, and *S. papillosum* growing on hummocks and *S. cuspidatum* and *S. recurvum* in hollows (Pancost et al., 2011). Birch and pinewood dominate in the surrounding dry areas. The average annual temperature at the site is 8.9°C (range 0.6 to 17.2°C) and pH in the range 3.8 to 4.3 (Charman et al., 2007). Water table depth is dynamic, and ranged between 0 and 56 cm (measured in late summer 2003; Charman et al., 2007), with a modern depth of 20 cm indicated by a reconstruction based on testate amoebae assemblages (Charman et al., 2007; Pancost et al., 2011). Despite having conservation status, the surface hydrology has been strongly affected by drainage, meaning that the shallow subsurface microbial assemblages could have been affected by the recent human activity at this peatland (Pancost et al., 2011). The core used in this study was 422 cm long and has been analysed for macrofossils and pollen, testate amoebae and humification indices as part of the ACCROTELM project (e.g. Yeloff et al., 2006; Charman et al., 2007; McClymont et al., 2008; Pancost et al., 2011). Macrofossils in the core have been dated, and calibrated ages are based on 'wiggle matched' AMS radiocarbon dates, using software for Bayesian age-depth modelling (Pancost et al., 2011 and references therein). Samples were stored at -20°C and were freeze-dried prior to analysis.

## 2.2 Total Organic Carbon (TOC) analysis

Aliquots were analysed in duplicate using a Carlo-Erba EA1108 elemental analyser to determine percentage carbon, nitrogen and hydrogen. Percentage inorganic carbon was determined using a Strohlein Instruments Coulomat 702 carbon analyser adapted to analyse $CO_2$ liberated from $H_3PO_4$ digestion. Elemental compositions were determined as percent of the dry weight of peat analysed. Total organic carbon was calculated as the

difference between total percentage carbon and total inorganic carbon (McClymont et al., 2008).

*2.3. Extraction and derivatisation,*

Lipids were extracted from approximately 0.1 - 0.3 g of freeze-dried, ground peat using repeated ultrasonication (x 3) with 5 ml of dichloromethane/methanol (1:1, v/v). This protocol was originally applied to recover and quantify neutral lipids with a focus on determining palaeoclimate information as part of the ACCROTELM project (McClymont et al., 2011, Pancost et al. 2011).Twenty two sample extracts were selected for BHP analysis via LCMS. After addition of the internal standard (5β-pregnane-3α,20α-diol), aliquots of the TLE were analysed as acetates, formed by heating with acetic anhydride/pyridine (4 ml; 1:1 v/v) at 50°C for 1 h and leaving at room temperature overnight. The derivatised extract was rotary evaporated to dryness and redissolved in 500 µL MeOH/propan-2-ol (6:4 v/v) for LC-MS analysis.

*2.4. LC-MS analysis*

Reversed-phase HPLC was performed using a Surveyor HPLC system (ThermoFinnigan, Hemel Hempstead, UK) fitted with a Phenomenex (Macclesfield, UK) Gemini $C_{18}$ 5 µm column (150 mm x 3.0 mm i.d.) and a security guard column cartridge of the same material. Separation was achieved at 30ºC with a flow-rate of 0.5 mL $min^{-1}$ and the following gradient profile: 90% A and 10% B (0 min); 59% A, 1% B and 40% C (at 25 min), then isocratic to 45 min, returning to the starting conditions in 5 min and stabilising for 10 min before injecting the next sample (A = MeOH, B = water, C = propan-2-ol; all HPLC grade from Thermo Fisher Scientific).

LC-$MS^n$ was performed using a Finnigan LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source operated in positive ion mode. LC-MS settings were as follows: capillary 155°C, APCI vaporiser 490°C, corona discharge current 8 μA, sheath gas flow 40 and auxiliary gas 10 (arbitrary units). LCQ instrument parameters were selected using an automated tune facility on a direct infusion of an acetylated standard of bacteriohopanetetrol cyclitol ether on the protonated molecular ion, *m/z* 1002 ($[M+H]^+$). LC-$MS^n$ analysis was carried out in data-dependent mode with three scan events: SCAN 1 – full mass spectrum, *m/z* 300-1300; SCAN 2: data-dependent $MS^2$ spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent $MS^3$ spectrum of most intense ion from SCAN 2. Detection was achieved at an isolation width of *m/z* 5.0 and fragmentation with normalised collisional dissociation energy of 35% and an activation Q value (parameter determining the *m/z* range of the observed fragment ions) of 0.15.

Structures assigned are based on comparison with authentic standards and published spectra where possible (Talbot et al., 2003a,b, 2007a,b, 2008) or by comparison of APCI $MS^2$ and $MS^3$ spectra with those of known compounds, as indicated below. The location of additional ring system methylation can be determined from relative retention times, with C-2 and C-3 methylated structures eluting ca. 0.7 and 1.3 min, respectively, after non-methylated compounds.

A semi-quantitative estimate of BHP abundance (±20%) is calculated from the characteristic base peak ion peak areas of individual BHPs in mass chromatograms (from SCAN 1) relative to the *m/z* 345 ($[M+H-CH_3COOH]^+$) base peak area response of the acetylated 5α-pregnane-3β,20β-diol internal standard. Averaged relative response factors (from a suite of five acetylated authentic BHP standards) are used to adjust the BHP peak areas relative to that of the internal standard where BHPs containing one or more nitrogen atoms give an averaged response approximately 12 times that of the standard and

compounds with no nitrogen atoms give a response approximately 8 times that of the standard (van Winden et al., 2012a).

## 3. Results

### *3.1 TOC*

TOC (%) contents in the upper section of the core (0-150 cm) are relatively stable, with a mean value of 43.4% ± 1.34 (± 1 standard deviation; min. 40.2%, max, 45.7%, n = 73). In the deeper section (350 – 422 cm), TOC contents are generally higher although more variable with an average of 46.1% ± 2.5%, (min 39.1%, max 49.1%, n = 31).

### *3.2 Bacteriohopanepolyols (BHPs)*

A total of 23 different BHPs (Tables 1 and 2) were identified in the 22 peat samples investigated, with each individual sample containing between 13 and 22 BHPs (Table 2). Total BHP abundance ranged from 150 to 2800 μg $g^{-1}_{TOC}$ (Table 2). The highest concentrations of BHPs were present in the 2-4 cm and 26-28 cm layers, within the region of water table fluctuation (0- 56 cm) and also in the four deepest layers (> 400 cm).

The total BHP concentration (Table 2) and those of major individual BHPs (Fig. 1, 2) show a similar depth profile, with highest values in the 2-4 cm, 26-28 cm and deepest samples (>400 cm) but with consistently low-to-intermediate values throughout the rest of the profile (e.g. Fig. 1, 2). The distributions are dominated by saturated tetrafunctionalised BHPs which account for over 80% of the total BHPs in all samples from below 10 cm. Three compounds in particular comprise the bulk of the total tetrafunctionalised BHPs: BHT (**Ia;** Fig. 1a), BHT cyclitol ether (**Ij** and/or **Ik;** Fig. 1b) and aminotriol (**Id**; Fig. 1c)

accounting for over 55% of the total BHPs in all except the surface and 4-6 cm samples. Several other less abundant BHPs do not follow this general trend as discussed below.

*3.2.1 35-amino functionalised BHPs*

Aminotriol (**Id**; Fig. 1c) was the most abundant compound in 14 of the 22 samples. It always co-occurred with aminotetrol (**Ie**; Fig. 1d), although aminotriol is consistently and significantly more abundant (up to 910 μg $g^{-1}_{TOC}$ compared to <40 μg $g^{-1}_{TOC}$ for aminotetrol; Table 2). The related hexafunctionalised aminopentol (**If**; Fig. 1e), was only observed below 22 cm and was the only compound identified which showed this profile. Where present, it only occurred in very low concentrations <5 μg $g^{-1}_{TOC}$ (Fig. 1e; Table 2) and never represented more than 0.5% of the total BHPs. Two minor methylated aminotriols were also observed, 2-methyl and 3-methyl-aminotriol (**IIb** and **IIIb;** Table 2).

*3.2.2 Composite BHPs*

With the exception of the surface and 4-6 cm samples, the composite structure BHT cyclitol ether (**Ij** and **Ik**; Fig. 1b) was the most abundant or 2$^{nd}$ most abundant compound in all samples (Table 2). The isomeric compound BHT glycoside (**Im**; Table 2) was also present in all but one sample but at significantly lower abundance, similar to the saturated bacteriohopanepentol- and bacteriohopanehexol-cyclitol ethers (**In** and **Io**, respectively; Table 2). A novel composite non-methylated hexafunctionalised BHP with a previously unrecognised terminal group structure (indicated by ion of m/z 344 in $MS^2$ spectrum of *m/z* 1132; data not shown) was also observed in the surface samples and below 400 cm, but was only rarely observed in the upper section (0-130 cm; Table 2).

*3.2.3 Unsaturated BHPs*

The surface and 4-6 cm samples had unusual BHP signatures, dominated by unsaturated compounds. These compounds are also present in significant amounts in the 2-4 and 8-10 cm samples (Fig. 2a,b; Table 2). A pair of peaks in the *m/z* 653 mass chromatograms have mass spectra consistent with the previously reported spectra of monounsaturated tetrols (cf. Talbot et al., 2007b, 2008). An unsaturated aminotriol (**IVb** or **Vb** or with unsaturation in the side chain [van Winden et al., 2012a]; Table 2) was also observed, primarily in the shallower sections. Also present are two unsaturated composite BHP compounds, including an unsaturated bacteriohopanepentol cyclitol ether (**IVn** or **Vn**; Table 2). The second is a composite tetrafunctionalised BHP, previously proposed to contain a pentose terminal group (**IVi** or **Vi**, Fig. 2b; Talbot et al., 2008). The unsaturated BHT pentose is particularly dominant in the surface sample, comprising 37% of total BHPs; however, its abundance, both total and relative to other BHPs, decreases rapidly with depth down core (Fig. 2b; Table 2). The related saturated homologue (**Ii;** Fig. 2c) also had its highest relative abundance in the surface layers. However, unlike the unsaturated structure (**IVi** or **Vi**; Fig. 2b), it was present throughout the core, with highest concentration in the deepest layers (>402 cm).

All of the aforementioned unsaturated BHPs exhibit markedly similar depth profiles. Maximum concentrations and relative abundances occur in the three most shallow horizons and then decrease dramatically down core and are not detected throughout most of the intermediate depths down to 120 cm (Fig. 2a,b). However, in the deepest part of the core, at >400 cm, these compounds are again present, although at lower relative abundance than the most shallow horizons. This is due to an even greater increase in concentration of other compounds including BHT, Aminotriol and BHT cyclitol ether (Fig. 1), but still indicates good preservation of unsaturated compounds at these depths (Fig. 2).

*3.2.4 Adenosylhopane and related structures*

Adenosylhopane (**Ig**; Fig. 2d) is always the most abundant representative of the group containing a cyclised side chain and collectively known as “soil-marker BHPs” (Cooke et al., 2008a; Zhu et al., 2011; Doğrul Selver et al., 2012). Adenosylhopane, a related structure (“adenosylhopane Type 2”) with the same cyclised side chain but an alternative terminal group, and two methylated homologues (**Ih**; **IIg**, **IIh** respectively; combined sum in Fig. 2e) all exhibited similar depth profiles, being most abundant in the 2-4 and 8-10 and 26-28 cm samples (Fig. 2e; Table 2). These compounds represent up to a maximum of 16% (Fig. 3a) then fall as low as 1.7% and remain low throughout the rest of the core (Fig. 3a).

*3.2.5 Methylated BHPs*

Six different methylated BHPs were detected in the peat, five of which were methylated at the C-2 position. These compounds represent between 2 and 12 % of the total BHPs in each sample (Table 2). 2-methyl BHT (**IIa**; Table 2) is present in all samples, and 2-methylaminotriol (**IId**; Table 2) is present in all but four of the near surface samples. The three other C-2 methylated BHPs had a much more limited occurrence, with the methylated adenosylhopane and the methylated homologue of the related compound (**IIg**, **IIh**; Table 2) only present in a few of the surface samples above 22 cm depth. The fifth compound, 2-methylBHT pseudopentose (**IIi**), was only detected in 3 samples below 400 cm and in very low abundance (Table 2). Finally, one C-3 methylated compound was observed, 3-methyl-aminotriol (**IIIb**); however, it was only present below 18 cm depth and always at low levels (Table 2), similar to the concentration profile of aminopentol (**If**; Fig. 1e).

## 4. Discussion

328

329 4.1 Sources of BHPs in BM peat

330 Microbiological studies of *Sphagnum* and peats reveal both typical bacterial groups
331 but also pronounced variations in distribution that appear to depend largely on abiotic
332 factors such as pH (e.g. Bragina et al., 2012a). Particularly common organisms include
333 Alphaproteobacteria with subordinate contributions from Beta-, Gamma- and in some
334 cases Deltaproteobacteria (e.g. Dedysh et al., 2006; Bragina et al., 2012a, b; Serkebaeva
335 et al., 2013). Many of these bacteria are heterotrophic and some are also capable of
336 dinitrogen fixation. In a recent study of a peat soil targeting the *nifH* gene, one of the main
337 components of the nitrogenase complex, a high diversity of diazotrophic bacteria were
338 detected (Zadorina et al., 2009). These sequences also included, but were not limited to,
339 species of Alpha-, Beta-, Gamma and Deltaproteobacteria. Other important phyla in the
340 peat microbiome include Acidobacteria, Actinobacteria, Planctomycetes and
341 Verrucomicrobia (Dedysh et al., 2006; Bragina et al., 2012b; Serkebaeva et al., 2013).
342 Each of these phyla are known to include hopanoid producers (Table 1; e.g. Rohmer et al.,
343 1984; Pearson et al., 2007), although biosynthesis by members of the proteobacteria has
344 been studied much more extensively than most of the other, more recently described phyla
345 (Rohmer et al., 1984; Ourisson et al., 1987; Farrimond et al., 1998; Kuchta et al., 1998;
346 Talbot et al., 2008; see also references in Table 1).

347

348 4.1.1 Heterotrophs

349 As BHP abundances are high in the shallowest layers (Table 2; Figs 1-3) it is
350 likely that many of the most abundant BHPs derive from aerobic heterotrophs. These
351 organisms are important members of the bacterial community found in peat, consuming a
352 wide range of organic substrates, including organic acids, sugars, polyalcohols and some
353 aromatic compounds as carbon and energy sources (e.g. Belova et al., 2006). This is

354 consistent with previous studies on peats which suggest that $^{13}$C-enriched hopanes are an
355 indicator of a heterotrophic bacterial population consuming $^{13}$C-enriched carbohydrates
356 (Pancost et al., 2000; Xie et al., 2004).

357 The BHP profile of BM is broadly consistent with a dominantly heterotrophic
358 bacterial community. Most heterotrophs make BHT (**Ia**), and the majority also make
359 composite BHPs, with BHT cyclitol ether (**Ij** and/or **Ik**) the most commonly-occurring
360 structure found in members of the Alpha-, Beta-, Gamma and Deltaproteobacteria (see
361 Table 1). Members of the Betaproteobacterial genus *Burkholderia*, which comprises gram-
362 negative, aerobic and microaerophilic chemoorganotrophic bacteria, have been shown to
363 be particularly important and abundant in the peat microbiome (Opelt and Berg, 2004;
364 Belova et al., 2006; Opelt et al., 2007; Sun et al., 2014) and are likely to be important
365 sources of both BHT and cyclitol ether compounds (Table 1). A high diversity of
366 *Burkholderia* species was found in both the endophytic and ectophytic habitats of different
367 *Sphagnum* moss species and in relation to two species (*S. magellanicum*, Opelt et al.,
368 2007; *S. rubellum,* Opelt and Berg, 2004) that are also present at BM.

369 The *Acetobacter* and *Gluconacetobacter* genera are known as acetic acid bacteria
370 (AAB). They are diazotrophic (e.g. Bragina et al., 2012b) and also produce acetate which
371 is a necessary substrate for acetoclastic methanogenesis which is an important pathway in
372 some shallow peats (Kelley et al., 1992; Popp and Chanton, 1999; Chasar et al., 2000;
373 Metje and Frenzel, 2005). Alphaproteobacteria AAB are prolific sources of a wide range of
374 BHPs with core structures of BHT (**Ia**), BHT cyclitol ether (**Ij**) and BHpentol cyclitol ether
375 (**In**) but also including mono and diunsaturated compounds with double bonds at C-6 (**IV**),
376 C-11(**V**) or both (**VI**) and with ring systems both with and without additional methylation at
377 C-3 (**III**; Rohmer and Ourisson, 1986; Peiseler and Rohmer, 1992; Talbot et al., 2007b).
378 Whilst AAB are likely present in BM and a possible source for the numerous unsaturated

BHPs, the absence of any C-3 methylated homologues, either of the tetrol or tetra- or pentafunctionalised cyclitol ethers, suggests contributions from these organisms is minor.

Of the species of Deltaproteobacteria investigated, only *Geobacter* species are considered potential sources here (Table 1); however, the absence of guanidine substituted BHT cyclitol ether which co-occurs with BHT, BHT cyclitol ether and BHT glucosamine in *Geobacter* spp. suggests a contribution from these organisms is unlikely or limited at this site. Other genomic studies have shown that *sqhC* diversity related to as yet unknown Deltaproteobacteria is high, especially in soils relative to marine sediments (Pearson et al., 2009), such that contributions from other Deltaproteobacteria cannot be excluded.

Amongst the heterotrophs, relatively few species make only non-composite BHPs. The Alphaproteobacterial genera *Beijerinkia* and *Bradyrhizobium* contain relatively simple BHP distributions dominated by aminotriol (**Id**), with additional BHT (**Ia**) in *Beijerinkia indica* (Table 1). Members of the Actinobacteria also only make these less complex compounds (Table 1). However, as these compounds represent two of the three most abundant compounds at BM (Fig. 1; Table 2) contributions from such sources could be important. Unlike any of the other heterotrophs with known BHP compositions, *Bradyrhizobium japonicum* also accumulates adenosylhopane (**Ig**; Table 1), which is present in the highest concentration in the 2-4 cm sample and constituted the greatest relative proportion of the total BHPs (13.4%; Table 2) in the 10-12 cm sample, likely indicating an aerobic source such as *B. japonicum*. Adenosylhopane is a biosynthetic intermediate in the synthesis of other side chain elongated BHPs (Bradley et al., 2010); therefore, there are potentially numerous sources for this compound, although, with the exception of *B. japonicum* few organisms have been shown to accumulate it in detectable amounts (Table 1).

404 To summarise, proteobacteria are undoubtedly significant sources of BHPs at BM,
405 with *Burkholderia* spp. considered particularly important sources of BHT (**Ia**) and BHT
406 cyclitol (**Ij** and/or **Ik**) although multiple sources are expected for both compounds (see
407 Table 1). *Beijerinckia* spp. are likely sources of aminotriol (**Id)** with additional contributions
408 from *Bradyhizobium* sp. and Actinobacteria. *B. Japonicum* likely also contributes to
409 adenosylhopane (**Ig**).
410
411 *4.1.2 Aerobic methane oxidising bacteria (methanotrophs)*
412 The northern peatlands cover an area of approximately 4 million $km^2$ (>45°N; Yu et
413 al., 2010 and references therein), and are a major source of methane release to the
414 atmosphere (e.g. Spahni et al., 2011 and references therein). Recent estimates vary but
415 indicate the flux of methane from northern peatlands is in the range 24 to 58 Tg $CH_4$ $yr^{-1}$
416 (Zhang et al., 2016 and references therein). This release is attenuated by methane
417 consuming bacteria (methanotrophs) inhabiting the oxic layers of the peat (e.g. Segers,
418 1998; Dedysh, 2009) and within *Sphagnum* (Raghoebarsing et al., 2005). Culturable
419 methanotrophs are classified phylogenetically as either members of the
420 Alphaproteobacteria (known as Type II) or Gammaproteobacteria (known as Type I;
421 Hanson and Hanson, 1996), with a third group recently described from the phylum
422 Verrucomicrobia (Op Den Camp et al., 2009).
423 Dedysh (2009) described the methanotroph diversity in acidic northern wetlands
424 and found that these settings are mainly colonized by methanotrophic representatives of
425 the Alphaproteobacteria (i.e. Type II methanotrophs). However, studies using functional
426 gene analysis by microarray or ultra-deep pyrosequencing of *pmoA* genes on *Sphagnum*
427 peat samples from a range of environments including but not limited to northern regions
428 (Siberia, Sweden, Canada, Argentina and The Netherlands), revealed Type I organisms
429 including the genera *Methylomonas, Methylobacter*, *Methylomicrobium* and

430 *Methylocaldum* (Kip et al., 2010, 2011). Type II organisms related to *Methylocystis* and
431 *Methylosinus* spp. (family Methylocystaceae) were also significant members of the
432 methanotroph community at all sites (Kip et al., 2010, 2011) and were particularly
433 dominant in *Sphagnum magellanicum* dominated habitats from Patagonia (Kip et al.,
434 2012). Whilst *Methylocystis* spp. are a common and abundant group in acidic peats, there
435 are other more recently described genera of Type II methanotrophs from the family
436 *Beijerinckiaceae* including *Methylocella* and *Methylocapsa*, which are also important and
437 would not have been observed in some earlier studies utilising the functional gene *pmoA*
438 (which is not present in these organisms; e.g. Rahman et al., 2011 and references
439 therein). Recent studies have also suggested an apparently symbiotic relationship
440 between methanotrophs and *Sphagnum* moss (Kip et al., 2010), with a bacterium
441 occurring inside cells of *S. cuspidatum* showing 93% 16S rRNA sequence similarity to
442 cultured *Methylocella* and *Methylocapsa* sp. (Raghoebarsing et al., 2005).

443 Previous studies of methane oxidising bacteria have shown that they produce
444 characteristic non-composite BHP distributions, with most Type I species producing high
445 levels of the hexafunctionalised compound aminopentol (**If**) and lower amounts of the
446 related pentafunctionalised compound aminotetrol (**Ie**; Table 1; e.g. Talbot et al., 2001; van
447 Winden et al., 2012a; Talbot et al., 2014 and references therein). Some Type I organisms
448 of the genera *Methylococcus* and *Methylocaldum* also produce homologues of
449 aminopentol and aminotetrol with a methyl group at position C-3 (**IIIf** and **IIIe** respectively;
450 Neunlist and Rohmer, 1985a; Cvejic et al., 2000a), although the 3-methyl-aminotriol (**IIIb**)
451 observed here (Table 2) has only recently been reported from cultures of the Type I
452 organism *Methylomicrobium alcaliphilum* (Banta et al., 2015), an alkaliphilic organism
453 unlikely to be present in *Sphagnum* peat. Type II organisms, including representatives of
454 the generas *Methylocystis* and *Methylosinus* (Alphaproteobacteria), produce a
455 combination of BHT, aminotriol and aminotetrol (Rohmer et al., 1984; Neunlist and

456 Rohmer, 1985b; Cvejic et al., 2000a; Talbot et al., 2001; van Winden et al., 2012a). It
457 should be noted, however, that there are multiple other sources of aminotriol especially
458 amongst other Alphaproteobacteria (Table 1; e.g. Talbot and Farrimond, 2007 and
459 references therein).

460 Of the other Type II methanotrophs from the family Beijerinckiaceae, no cultured
461 representatives of *Methylocapsa* sp. (e.g. Dedysh et al., 2001a) have been tested for BHP
462 production or composition, but van Winden et al. (2012a) reported the BHP composition of
463 *Methylocella palustris. Methylocella* and *Methylocapsa* are closely related to another
464 known BHP producer *Beijerinckia* sp., which makes aminotriol (**Id**) and in some cases BHT
465 (**Ia**; Table 1; Vilcheze et al., 1994) but they were not found to produce aminotetrol (**Ie**). The
466 dominant compounds in *M. palustris* (and also *Methylocella tundrae*; Talbot and Rohmer,
467 unpublished data) were aminotriol and BHT with trace levels of adenosylhopane (**Ig**).
468 Unfortunately, there are numerous different sources for all of these compounds so there is
469 no way to conclusively identify BHP contributions from *Methylocella* spp. using BHP
470 analysis (Table 1). Given the importance of this group as demonstrated by microbiological
471 studies (Kip et al., 2010, 2011; Rahman et al., 2011), we consider it likely that Type II
472 methanotrophs of the Beijerinckiaceae family will be important sources of (at least)
473 aminotriol (**Id**) at this site.

474 Although a methanotroph biomarker might be expected to be most abundant at the
475 redox interface i.e. water table depth, where oxygen is present and methane
476 concentrations are the highest (e.g. Sundh et al., 1995), aminotriol was present at high
477 concentration in all samples from BM (Fig. 1c). Aminotetrol (**Ie**) was also present in all
478 samples, representing up to 2.3% of the total BHP distribution with the highest
479 concentration in the 26-28 cm sample (Fig. 1d). Although this is around the approximate
480 average depth of the oxic-anoxic interface, this region of the core will be exposed to
481 oxygen seasonally as the water table lowers. The highest concentration of aminopentol (**If**)

for the entire profile occurred in the same sample (Table 2), and it was only present below 22 cm; however, it never represented more than 0.4% of the total BHP distribution (Fig. 1e). The occurrence of aminotriol and aminotetrol together, in the absence of aminopentol, in the upper section of the core (0 to 22cm) suggests the presence of Type II methanotrophs *Methylocystis/Methylosinus* (family Methylocystaceae; Table 1) at BM which is consistent with previous studies on methanotroph populations in peat (e.g. Dedysh et al., 2001b; Dedysh, 2009; Kip et al., 2011).

Aminopentol (**If**), which is only known to occur in Type I methane oxidising bacteria (see review in Talbot et al., 2014), was only observed below 22 cm depth (Fig. 1e). Although this depth is within the reported range of water table variations (0-56 cm; Charman et al., 2007), it is below the current reconstructed water table level (~20 cm) based on testate amoebae analysis (Pancost et al., 2011 after Charman et al., 2007). This suggests that the occurrence of aminopentol in deeper samples likely reflects the presence of relict BHPs initially formed at the anoxic-oxic interface. As such, aminopentol is most likely a fossil compound recording past methanotrophy. The absence of C-3 methylated homologues of aminopentol and aminotetrol suggest a likely source organism would be *Methylomonas* sp., consistent with the identification of this genera in *Sphagnum* peat in other studies (Kip et al., 2010) and the recent isolation of the first acid tolerant *Methylomonas* sp. from and acidic *Sphagnum* peat bog (Danilova et al., 2013).

We speculate that the only C-3 methylated BHP observed at BM, 3-methylaminotriol (**IIId**; Table 2), present from 18 cm depth and below is most likely related to a genera of Type I methanotrophs based on the similar depth profile to that of aminopentol (which occurs from 22 cm and below; Fig. 1a; Table 2).

Aminotriol concentrations are over two orders of magnitude greater than aminotetrol concentrations, inconsistent with their relative abundances in previously cultured type II methanotrophs (Neunlist and Rohmer, 1985b; Jahnke et al., 1999; Talbot et al., 2001; van

508 Winden et al., 2012a). Instead, the marked similarity of the aminotriol BHP depth profile
509 with most other major BHPs (Fig. 1) suggests a significant non-methanotrophic origin for
510 this compound, or a methanotroph origin from one of these more recently described
511 sources (e.g. *Methylocella*; van Winden et al., 2012a). The similar depth profile of
512 aminotetrol, although far less abundant (Fig. 1c and d), suggests that this might also
513 derive, at least partially, from other sources, although the only known non-methanotroph
514 source are sulphate reducing bacteria of the genus *Desulfovibrio* (Blumenberg al., 2006,
515 2009b, 2012), which is unlikely to occur in this environment. Only aminopentol has a depth
516 profile consistent with being derived predominantly from methanotrophs.

517 Finally, although anaerobic methane-oxidisers related to the novel bacterium
518 "*Candidatus* Methylomirabillis oxyfera" have been identified in peat (e.g. Zhu et al., 2012),
519 the diagnostic BHP produced by these organisms (3-Methyl-bacteriohopanehexol, **IIIf;**
520 Kool et al., 2014) was not detected.

521

522 *4.1.3 Phototrophic bacteria*

523 The surface layers of peat bogs host a wide range of phototrophic eukaryotes
524 and prokaryotes. This includes nitrogen fixing cyanobacteria which inhabit *Sphagnum* peat
525 bogs, occurring in both epiphytic and intracellular associations with *Sphagnum*, (e.g.
526 Granhall and Selander, 1973; Krivograd Klemenčič and Vrhovška, 2003; Krivograd
527 Klemenčič et al., 2010; Bragina et al., 2012b). Many different classes of hopanoid
528 producing cyanobacteria, including *Anabaena*, *Calothrix*, *Cyanothece*, *Gloeocapsa*,
529 *Microcystis*, *Nostoc*, *Oscillatoria* and *Phormidium* (Talbot et al., 2008 and references
530 therein), have been observed in peat (Krivograd Klemenčič et al., 2010 and references
531 therein). Until recently, cyanobacteria were considered to be the major source of
532 hopanoids methylated at the C-2 position (Summons et al., 1999); however, recent
533 genomic studies have revealed that the capacity for this synthesis also occurs in other

534 phyla, particularly the Alphaproteobacteria and also Acidobacteria (Welander et al., 2010)
535 and that it is a particularly common trait in organisms found in close (symbiotic)
536 association with plants (Ricci et al., 2014).

537 Of particular interest to this study are the species of *Gloeocapsa* cyanobacteria
538 which were identified, for example, in the Männikjärve bog (central Estonia) and were
539 particularly abundant on *S. magellanicum* plants (Karofeld and Toom, 1999), one of the
540 species present at the BM site. A further seven *Gloeocapsa* spp. were identified in two
541 Slovenian bogs (Krivograd Klemenčič and Vrhovška, 2003). Crucially, a *Gloeocapsa* sp. is
542 the only known source of the unsaturated compound, identified as a "BHT pentose" (**IVi** or
543 **Vi**), that is highly abundant (over 40%) in the surface layers at the BM site (Fig. 1b). Such
544 a high abundance in surface layers is consistent with an aerobic and/or phototrophic
545 source and suggests a particular ecophysiological role, potentially regulating osmotic
546 pressure or proton gradients in the low pH peat environment (cf. Welander et al., 2009).
547 The original identification of this compound, together with its saturated and C-2 methylated
548 homologues (**IIi**) was from a sample of an epilithic colony of *Gloeocapsa* sp. from Devon
549 Island (Arctic; Talbot et al., 2008). The saturated pentose compound, with the non-
550 methylated structure (**Ii**, Table 2, Fig. 2c), was also observed in BM peat, being present at
551 all depths but showing a markedly different depth profile to the unsaturated structure (Fig.
552 2b). The methylated pentose structure (**IIi**; Table 2) was only observed in the deepest
553 levels, below 400 cm. Therefore, the saturated compounds could: (i) derive from an
554 additional source to the unsaturated compounds; (ii) have a markedly different
555 preservation potential for the methylated vs non-methylated compounds; and/or (iii) have
556 different extraction efficiencies in the fresher, near surface material, given that some other
557 methylated BHPs have exhibited resistance to extraction (Herrmann et al., 1996; Allen et
558 al., 2010).

559 The pair of unsaturated bacteriohopanetetrols (**IVa**, **b** or **c**; Table 2) also had very
560 similar depth profiles to the unsaturated BHT-carbopseudopentose suggesting a common
561 source (Fig. 2a and b). A single unsaturated BHT with an identical mass spectrum was
562 also observed in the original source of the *Gloeocapsa* enrichment (Talbot et al., 2008).
563 One possibility, therefore, is that a *Gloeocapsa* sp. inhabiting this environment produces
564 both the composite carbopseudopentose and one or both of the observed unsaturated
565 tetrols. An alternative heterotrophic source for the unsaturated tetrols, the
566 Alphaproteobacteria AAB, are discussed above but considered minor at most, again due
567 to the absence of C-3 methylated homologues.
568 Purple non-sulfur bacteria (PNSB) are normally anoxygenic photoheterotrophic
569 organisms. They belong to the Alphaproteobacteria and Betaproteobacteria with many
570 representatives being closely related to non-phototrophic, strictly chemotrophic bacteria
571 (Kulichevskaya et al., 2006 and references therein). PNSB are widely distributed in various
572 aquatic ecosystems as well as in sediments, moist soils and natural wetlands
573 (Kulichevskaya et al., 2006), but peat bogs are rarely reported as sources of PNSB
574 (Kulichevskaya et al., 2006). Few PNSB can tolerate low pH with the exception of
575 members of the genera *Rhodoblastus* (Pfennig, 1969; Imhoff, 2001). Several species of
576 *Rhodoblastus* have been isolated from acidic *Sphagnum* bogs (Kulichevskaya et al.,
577 2006), and *Rhodoblastus acidophilus* (formerly *Rhodopseudomonas acidophila*; Imhoff
578 2001) has been shown to produce a number of common hopanoids including BHT (**Ia**) and
579 BHT cyclitol ether (**Ij**; Talbot et al., 2007a and references therein). The major product,
580 however, was adenosylhopane (**Ig**, Table 1; also seen in other PNSB including
581 *Rhodopseudomonas*; Talbot et al., 2007a and references therein; Eickhoff et al., 2013a)
582 suggesting another possible source of adenosylhopane at BM.
583
584 ***4.2 Comparison of BHP distributions at BM to other soils and peat***

585 Biohopanoids were present at high concentration throughout the BM peat core (Table 2). A
586 total of 23 different intact BHP structures were observed (Table 2), comparable to recent
587 reports of BHP distributions in soils (e.g. Cooke et al., 2008a; Xu et al., 2009; Zhu et al.,
588 2011; Spencer-Jones et al., 2015) and considerably more complex than typical
589 distributions in aquatic sediments (e.g. Blumenberg et al, 2006, 2009a, 2010; Talbot and
590 Farrimond, 2007; Coolen et al., 2008; Cooke et al., 2008b, 2009), with the exception of
591 sediments occurring on deep-sea fans receiving high terrigenous inputs (Handley et al.,
592 2010; Wagner et al., 2014). Although the extraction method used here (sonication in
593 DCM:MeOH) was not the typical modified Bligh and Dyer method (e.g. Cooke et al.,
594 2008a), which has been widely applied to BHP extractions in other studies, the BHP
595 distributions are comparable and the concentrations in many cases are similar or higher
596 than those reported for other peats (Kim et al., 2011; van Winden et al., 2012a,b). The
597 highest total BHP concentrations were found in the 2-4 cm sample (2700 $\mu g\ g^{-1}_{TOC}$) and
598 below 400 cm (1100-3000 $\mu g\ g^{-1}_{TOC}$), whilst values throughout the remainder of the core
599 were lower, ranging from 160 $\mu g\ g^{-1}_{TOC}$ (32-34 cm) to 900 $\mu g\ g^{-1}_{TOC}$ (Table 2).

600 Although to date BHPs in peat have only been studied by LCMS from 3 other
601 locations (Kim et al., 2011; van Winden et al., 2012a,b), our data collectively suggest that
602 whilst soil and peat derived organic matter generally contain the same BHPs, they have
603 characteristically different relative distributions, as recently suggested for tropical and non-
604 tropical soils (Spencer-Jones et al., 2015). Excluding the surface layers, the three most
605 dominant BHPs in the BM peat at all other depths were BHT (**Ia**), BHT-cyclitol ether (**Ij**)
606 and aminotriol (**Id**). Again, these observations are consistent with other recent studies of
607 BHPs in peat (Kim et al., 2011; van Winden et al., 2012a,b) and also with the BHP
608 composition in soils from the Northern hemisphere (Cooke et al., 2008a; Xu et al., 2009;
609 Cooke, 2010; Rethemeyer et al., 2010; Kim et al., 2011) and tropical settings (Wagner et
610 al., 2014; Spencer-Jones et al., 2015). However, we find that the BHP distribution at BM,

and other peats, differs from soils in two fundamental ways: (i) they contain a particularly high proportion of unsaturated BHPs in peat surface layers; and (ii) generally exhibit lower proportions of the 'soil-marker' BHPs.

The surface layers of the BM peat core contain exceptionally high concentrations of unsaturated biohopanoids, including unsaturated BHT-pentose (**IVi or Vi**), in agreement with observations by van Winden et al. (2012a) for a UK peat bog. The BM site also uniquely contained two isomers of unsaturated BHT (possibly **IVa** and/or **b** or **c**), whilst only one isomer was found in the UK study (van Winden et al., 2012a). These compounds, together with 2 other minor unsaturated BHPs (**IVd** or **Vd** and **IVm** or **Vm**) accounted for up to 46 % in the surface layers, rapidly falling to 3% or less below 12 cm depth (although all unsaturated BHPs were observed again below 400 cm depth; Fig. 2 a and b; Table 2).

Among the defining features of soil BHP distributions are high levels of adenosylhopane (**Ig**), its C-2 methylated homologue (**IIg**; Talbot et al., 2007a) and two pairs of related compounds with as yet unidentified terminal groups (including "adenosylhopane type 2"; **Ih** and **IIh**, Table 1), together comprising 28% of the average total BHP assemblage (Cooke et al., 2008a; Cooke, 2010). Five soils from Canada and permafrost soils from Svalbard and Siberia contained even higher proportions of this BHP group (Xu et al., 2009; Rethemeyer et al., 2010; Höfle et al., 2015). In the peat samples studied here, the distribution of these compounds is rather different, with adenosylhopane and related compounds only present in significant concentration in the shallow sub-surface (up to 15% in 2-4 and 8-12 cm; Fig. 3a) but are otherwise below 10%. In *Sphagnum* peats from 2 other European sites (UK, Belgium; van Winden et al., 2012a and b respectively), the relative abundance of soil marker BHPs is also low, typically less than 10% except in the near surface samples (up to 15%; Fig. 3a). The only exceptions are 4 peat samples from France which have a slightly higher relative abundance of soil biomarkers (20-30%;

636 Fig. 3b), however, no details were provided as to the type of peat in that study so it is
637 possible they were not *Sphagnum* peats (Kim et al., 2011).

638 The low values of soil marker BHPs (typically < 10%; Fig 3a,b) within peats are
639 distinctive from those of soils. Values reported recently for tropical and sub-tropical soils
640 had values in the range 0-40% although the majority were in the range 10-20%, slightly
641 higher than the peat samples (Fig. 3c). Temperate soils from Canada (Alberta), France
642 and the UK, had a wide range (0-70%; Fig. 3d) with the widest range found in permafrost
643 soils from the Siberian Arctic and Svalbard (0-90%; Fig. 3e), possibly reflecting competing
644 influences of low temperature leading to higher values and low pH leading to low values
645 (Höfle et al., 2015; Spencer-Jones et al., 2015). As discussed above, the most likely
646 sources of adenosylhopane (**Ig**) and related structures in *Sphagnum* peat environments
647 are nitrogen-fixing Alphaproteobacteria (*Bradyrhizobium* sp.; PNSB; *Methylocella* sp.;
648 Table 1). As adenosylhopane is in itself an intermediate in the biosynthetic pathway to
649 other side chain elongated BHPs (Bradley et al., 2010), this suggests that under the low
650 pH conditions found in *Sphagnum* peat (pH 3.8 – 4.3 at BM; Charman et al., 2007), the
651 species that would normally accumulate adenosylhopane convert this precursor to other
652 BHPs. Alternatively, other nitrogen fixers such as *Burkholderia* sp. (Betaproteobacteria)
653 and other, especially Alphaproteobacterial, BHP producers (e.g. Zadorina et al., 2009)
654 outcompete the adenosylhopane accumulators in these systems. In their study of BHPs in
655 Siberian permafrost, Höfle et al. (2015) reported that concentrations of adenosylhopane
656 (and related compounds) were negatively correlated with pH, the implication being that
657 further modification of the side chain is more important at lower pH than more neutral
658 conditions, hence the greater diversity of structures present in low pH peat and peaty soils
659 (Cooke et al., 2008a; van Winden et al., 2012a). Indeed Gong et al. (2015) recently
660 reported that pH was a key factor controlling the geographical distribution of squalene
661 hopene cyclase (*sqhC*) with Proteobacterial and Acidobacteria the dominant source

organisms in the acidic Dajiuhu peatland (China). Regardless of the reasons for this difference in BHP distributions, this variability could prove useful for tracing the origin and transport of terrestrially derived organic matter in the aquatic realm (Cooke et al., 2008b, 2009; Zhu et al., 2011; Doğrul Selver et al., 2012). Furthermore, these effects would almost certainly lead to variations in the values of the $R_{soil}$ and $R'_{soil}$ proxies with values at BM significantly lower than those reported recently for other soils (Table 2; see review in Spencer-Jones et al., 2015).

*4.3 Preservation of hopanoids at BM*

This is the first study to investigate the BHP distribution in peat samples to a depth of 410 cm, equivalent to an age of ~3000 cal. yr BP. We observe robust preservation of complex, highly functionalised BHPs at BM although there are reports of similarly complex hopanoids in marine sediments from the Congo deep sea fan dating to over 2.5 Ma (Handley et al., 2010; Talbot et al., 2014; Spencer-Jones, 2016) and the oldest confirmed biohopanoid is BHT, in ~50 Ma sediments from Tanzania (van Dongen et al., 2006).

Earlier studies on lignites, peats and soils have reported the rapid conversion of biohopanoids to geohopanoids (e.g. van Dorselaer et al., 1975; Quirk et al., 1984; Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995). That is also true here, with a significant increase in geohopanoid concentrations with depth (data not shown). Nonetheless, at BM we continue to see a full suite of 17β,21β(*H*) tetra-, penta- and hexafunctionalised BHPs even at >400 cm depth indicating favourable preservation conditions (Table 2). Intriguingly, the concentration of BHPs in the deepest samples (402-410 cm) are equivalent to those in the most concentrated near surface samples (2-4 cm; Table 2).

Although originally ascribed to a purely aerobic source, recent work has shown that there are also a number of potential obligate and facultative anaerobic sources that can produce BHPs, including Planctomycetes (Sinninghe Damsté et al., 2004; Rush et al.,

2014), *Desulfovibrio* sp. (Deltaproteobacteria; Blumenberg et al., 2012), *Rhodopseudomonas palustris* (Alphaproteobacteria; Rashby et al., 2007) and *Geobacter* sp. (Deltaproteobacteria; Fischer et al., 2005; Eickhoff et al., 2013b). The BHPs produced by members of these phyla and/or species are discussed above (see also Table 1); however, at this time few species from these groups can be directly related to peat environments. Therefore, although we cannot rule out some contribution from anaerobes at depth in the peat core, we propose that the major proportion of the BHPs are produced by aerobes at or above the water table and are subsequently preserved at depth.

## 5. Conclusions

A peat core from Bissendorfer Moor (Germany) contained a wide range of structurally distinct BHPs at high concentrations to a depth of 410 cm (c. 2900 cal. yr BP). By comparison with literature on *Sphagnum* peat microbiological communities, these lipids can be linked primarily to heterotrophic but also methanotrophic and phototrophic members of the peat microbiome. One of the most striking conclusions of this work, but one that is consistent with previous work, was the relatively small impact of bacterial methanotrophs on the BHP signature. Aminopentol, a biomarker for Type I methanotrophs, was only present below 22 cm but never represented more than 0.4% of the total BHP pool. Similarly aminotetrol, produced by both Type I and II methanotrophs, only accounted for up to 2% of total BHPs. This suggests that even in peat deposits, methanotrophs represent a relatively minor component of the bacterial (or at least the hopanoid-producing) population; settings where much higher proportions occur (e.g. Talbot et al., 2016) must be characterised by a particularly strong methane cycle. Collectively, the types of compounds present are similar to those reported from soils whilst the relative distributions show several distinct differences. The near surface samples contained

714 exceptionally high levels of a number of unsaturated BHPs including two isomers of
715 unsaturated BHT, a feature which has not been reported elsewhere. A second major
716 difference between BHPs in BM peat and those reported for soils was the relative
717 abundance of adenosylhopane and related structures, which was as high as 15% in a few
718 (near) surface horizons but generally below 10% and much lower (~1.7%) in deeper
719 layers. These values are significantly lower than those typically reported for soils from
720 other environments (tropical, temperate, Arctic) and are likely influenced by the low pH of
721 the peat environment.

722 BHP signatures in peat are unique and do appear to record specific peat
723 biogeochemical and ecological features, albeit with complex controls which are not yet
724 fully understood. They also have strong preservation potential such that they could be
725 useful in examining peat paleo-archives.

726

727 **Acknowledgements**

728 We thank Hansjörg Küster, Dmitri Mauquoy and Mareike Steffens for the collection
729 of the peat samples from Bissendorfer Moor, Germany. These samples were acquired
730 from the ACCROTELM Project, funded by the European Union Framework V under
731 contract number EVK2-CT-2002-00166. We are grateful for funding from the European
732 Research Council for a starting grant AMOPROX (258734) awarded to H.M.T. We thank
733 R. Berstan and I.D. Bull for technical support in the Organic Geochemistry Unit, School of
734 Chemistry, Bristol. We also thank The Science Research Investment Fund (SRIF) from
735 HEFCE for funding the ThermoFinnigan LCQ ion trap mass spectrometer (Newcastle).
736 RDP acknowledges the RS Wolfson Research Merit Award. RDP and GNI also
737 acknowledge the Advanced ERC Grant T-GRES (340923). Finally, we thank the associate
738 editor and two anonymous reviewers for their constructive comments.

739

## References


Allen, M.A., Neilan, B.A., Burns, B.P., Jahnke, L.L., Summons, R.E., 2010. Lipid biomarkers in Hamelin Pool microbial mats and stromatolites. Organic Geochemistry 41, 1207–1218.

Andersen, R., Chapman, S.J., Artz, R.R.E., 2013. Microbial communities in natural and disturbed peatlands: A review. Soil Biology and Biochemistry 57, 979-994.

Auman, A.J., Speake, C.C., Lidstrom, M.E., 2001. *nifH* sequences and nitrogen fixation in type I and type II methanotrophs. Applied and Environmental Microbiology 67, 4009–4016.

Banta, A., Wei, J.H., Welander, P.V., 2015. A distinct pathway for tetrahymanol synthesis in bacteria. Proceedings of the National Accademy of Science of the USA 112, 13478-13483.

Belova, S.E., Pankratov, T.A., Dedysh, S.N., 2006. Bacteria of the genus *Burkholderia* as a typical component of the microbial community of *Sphagnum* peat bogs. Microbiology 75, 90-96.

Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H.M., Oppermann, B.I., Seifert, R., Pape, T., Michaelis, W., 2006. Biosynthesis of hopanoids by sulfate-reducing bacteria (genus *Desulfovibrio*). Environmental Microbiology 8, 1220-1227.

Blumenberg, M., Seifert, R., Kasten, S., Bahlmann, E., Michaelis, W., 2009a. Euphotic zone bacterioplankton sources major sedimentary bacteriohopanepolyols in the Holocene Black Sea. Geochimica et Cosmochimica Acta 73, 750-766.

Blumenberg, M., Oppermann, B.I., Guyoneaud, R., Michaelis, W., 2009b. Hopanoid production by *Desulfovibrio bastinii* isolated from oilfield formation water. FEMS Microbiology Letters 293, 73–78.

Blumenberg, M., Mollenhauer, G., Zabel, M., Reimer, A., Thiel, V., 2010. Decoupling of bio- and geohopanoids in sediments of the Benguela Upwelling System (BUS). Organic Geochemistry 41, 1119–1129.

Blumenberg, M., Hoppert, M., Krüger, M., Dreier, A., Thiel, V., 2012. Novel findings on hopanoid occurrences among sulfate reducing bacteria: is there a direct link to nitrogen fixation? Organic Geochemistry 49, 1–5.

Bradley, A.S., Pearson, A., Sáenz, J.P., Marx, C.J., 2010. Adenosylhopane: the first intermediate in hopanoid side chain biosynthesis. Organic Geochemistry 41, 1075–1081.

Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., Berg, G., 2012a. *Sphagnum* mosses harbour highly specific bacterial diversity during their whole lifecycle. The ISME Journal 6, 802-813.

Bragina, A., Maier, S., Berg, C., Müller, H., Chobot, V., Hadacek, F., Berg, G., 2012b. Similar diversity of Alphaproteobacteria and nitrogenase gene amplicons on two related *Sphagnum* mosses. Frontiers in Microbiology. 2, Article 275, doi: 10.3389/fmicb.2011.00275.

Bragina, A., Berg, C., Müller, H., Moser, D., Berg, G., 2013. Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. Nature Scientific Reports 3, 1955; DOI:10.1038/srep01955

Bravo, J-M., Perzel, M., Härtner, T., Kannenberg, E.L., Rohmer, M., 2001. Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium japonicum* USDA 110. European Journal of Biochemistry 268, 1323-1331.

Chambers, F.M., Booth, R.K., De Vleeschouwer, F., Lamentowicz, M., Le Roux, G., Mauquoy, D., Nichols, J.E., van Geel, B., 2012. Development and refinement of proxy-climate indicators from peats. Quaternary International 268, 21-33.

Charman, D.J., Blundell, A., and ACCROTELM Members, 2007. A new European testate amoebae transfer function for palaeohydrological reconstruction on ombrotrophic peatlands. Journal of Quaternary Science 22, 209–221.

Chasar, L.S., Chanton, J.P., Glaser, P.H., Siegel, D.I., Rivers, J.S., .2000. Radiocarbon and stable carbon isotopic evidence for transport and transformation of dissolved organic carbon, dissolved inorganic carbon, and $CH_4$ in a northern Minnesota peatland. Global Biogeochemical Cycles 14, 1095-1108.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., House, J.I., 2013. Carbon and Other Biogeochemical Cycles: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Change. in O. Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, Z. T. & M J.C. (eds), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Change. Cambridge University Press, Cambridge, Cambridge, United Kingdom and New York, NY, USA.

Cooke, M.P., 2010. The Role of Bacteriohopanepolyols as Biomarkers for Soil Bacterial Communities and Soil Derived Organic Matter (Ph.D. thesis). Newcastle University.

Cooke, M.P., Talbot, H.M., Farrimond, P., 2008a. Bacterial populations recorded in bacteriohopanepolyol distributions in soils from Northern England. Organic Geochemistry 39, 1347-1350.

Cooke, M.P., Talbot, H.M., Wagner, T., 2008b. Tracking soil organic carbon transport to continental margin sediments using soil-specific hopanoid biomarkers: a case study from the Congo fan area. Organic Geochemistry 39, 958-971.

Cooke, M.P., van Dongen, B.E., Talbot, H.M., Semiletov, I., Shakhova, N., Guo, L., Gustafson, Ö., 2009. Transport of terrestrial organic matter to the Arctic Ocean via the great Arctic rivers. Organic Geochemistry 40, 1151-1159.

Coolen, M.J.L., Talbot, H.M., Abbas, B., Ward, C., Schouten, S., Volkman, J.K., Sinninghe Damsté, J.S., 2008. Sources for sedimentary bacteriohopanepolyols as revealed by 16S rDNA stratigraphy. Environmental Microbiology 10, 1783-1803.

Cvejic, J.H., Bodrossy, L., Kovács, K.L., Rohmer, M., 2000a. Bacterial triterpenoids of the hopane series from the methanotrophic bacteria *Methylocaldum* spp.: phylogenetic implications and

first evidence for an unsaturated aminobacteriohopanepolyol. FEMS Microbiology Letters182, 361-365.

Cvejic, J.H., Rosa Putra, S., El-Beltagy, A., Hattori, R., Hattori, T., Rohmer, M., 2000b. Bacterial triterpenoids of the hopane series as biomarkers for the chemotaxonomy of *Burkholderia*, *Pseudomonas* and *Ralstonia* spp. FEMS Microbiology Letters 183, 295-299.

Danilova, O.V., Kulichevskaya, I.S., Rozova, O.N., Detkova, E.N., Bodelier, P.L.E., Trotsenko, Y.A., Dedysh, S.N., 2013. *Methylomonas paludis* sp. nov., the first acid tolerant member of the genus *Methylomonas*, from an acidic wetland. International Journal of Systematic and Evolutionary Biology 63, 2282-2289.

Dedysh, S.N., Horz, H.-P., Dunfield, P., Liesack, W., 2001a. A novel pmoA lineage represented by the acidophilic methanotrophic bacterium *Methylocapsa acidiphila* B2. Archives of Microbiology 177, 117-121.

Dedysh, S.N., Derakshani, M., Liesack, W., 2001b. Detection and enumeration of methanotrophs in acidic *Sphagnum* peat by 16S rRNA fluorescence in situ hybridization, including the use of newly developed oligonucleotide proves for *Methylocella palustris*. Applied and Environmental Microbiology 67, 4850-4857.

Dedysh, S.N., Pankratov, T.A., Belova, S.E., Kulichevskaya, I.S., Liesack, W., 2006. Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. Applied and Environmental Microbiology 72, 2110–2117.

Dedysh, S.N., 2009. Exploring methanotroph diversity in acidic northern wetlands: molecular and cultivation-based studies. Microbiology 78, 655-669.

Dehmer, J., 1993. Petrology and organic geochemistry of peat samples from a raised bog in Kalimantan (Borneo). Organic Geochemistry 20, 349-362.

Dehmer, J., 1995. Petrological and organic geochemical investigation of recent peats with known environments of deposition. International Journal of Coal Geology 28, 111-138.

Doğrul Selver, A., Talbot, H.M., Gustafsson, Ö, Boult, S., van Dongen, B.E., 2012. Soil organic matter transport along a sub-Arctic river-sea transect. Organic Geochemistry 51, 63-72.

Doğrul Selver, A., Sparkes, R.B., Bischoff, J., Talbot, H.M., Gustafsson, Ö., Semilitov, I., Dudarev, O., Boult, S., van Dongen, B.E., 2015. Distributions of bacterial and archaeal membrane lipids in surface sediments reflect differences in input and loss of terrestrial organic carbon along a cross-shelf Arctic transect. Organic Geochemistry 83-84, 16-26.

Doughty, D.M., Hunter, R.C., Summona, R.E., Newman, D.K., 2009. 2-Methylhopanoids are maximally produced in akinetes of *Nostoc punctiforme*: geobiological implications. Geobiology 7, 524-532.

Eickhoff, M., Birgel, D., Talbot, H.M., Peckmann, J., Kappler, A., 2013a. Oxidation of Fe(II) leads to increased C-2 methylation of pentacyclic triterpenoids in the anoxygenic phototrophic bacterium *Rhodopseudomonas palustris* strain TIE-1. Geobiology 11, 268-278.

Eickhoff, M., Birgel, D., Talbot, H.M., Peckmann, J., Kappler, A., 2013b. Bacteriohopanoid inventory of *Geobacter sulfurreducens* and *Geobacter metallireducens*. Organic Geochemistry 58, 107-114.

Farrimond, P., Fox, P.A., Innes, H.E., Miskin, I.P., Head, I.M., 1998. Bacterial sources of hopanoids in Recent sediments: improving our understanding of Ancient hopane biomarkers. Ancient Biomolecules 2, 147–166.

Flesch, G., Rohmer, M., 1988. Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/carbon linkage between a triterpene and D-ribose. European Journal of Biochemistry 175, 405-411.

Flesch, G., Rohmer, M., 1989. Prokaryotic triterpenoids. A novel hopanoid from the ethanol-producing bacterium *Zymomonas mobilis*. Biochemical Journal 262, 673-675.

Fischer, W.W., Summons, R.E., Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. Geobiology 3, 33–40.

Garcia Costas, A.M., Tsukatani, Y., Rijpstra, W.I., Schouten, S., Welander, P.V., Summons, R.E., Bryant, D.A., 2012. Identification of the Bacteriochlorophylls, carotenoids, quinones, lipids, and hopanoids of "*Candidatus* Chloracidobacterium thermophilum". Journal of Bacteriology 194, 1158-1168.

Gong, L., Wang, H., Xiang, X., Qiu, X., Liu, Q., Wang, R., Zhao, R., Wang, C., 2015. pH shaping the composition of sqhC-containing bacterial communities. Geomicrobiology Journal 32, 433-444.

Gorham, E., 1991. Northern peatlands: Role in the carbon cycle and probable responses to climatic warming. Ecological Applications 1, 182–195.

Granhall, U., Selander, H., 1973. Nitrogen fixation in a subarctic mire. Oikos 24, 8-15.

Handley, L., Talbot, H.M., Cooke, M.P., Anderson, K.E., Wagner, T., 2010. Diverse fully functionalised bacteriohopanepolyol distributions up to 1.2 Ma in sediments from the Congo deep-sea fan. Organic Geochemistry 41, 910-914.

Hanson, R.H., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiological Reviews 60, 439–471.

Herrmann, D., Bisseret, P., Connan, J., Rohmer, M., 1996. A non-extractable triterpenoid of the hopanoid series in *Acetobacter xylinum*. FEMS Microbiology Letters 135, 323-326.

Höfle, S., Kusch, S., Talbot, H.M., Mollenhauer, G., Zubrzycki, S., Burghardt, S., Rethemeyer, J. 2015. Characterization of bacterial populations in Arctic permafrost soils using bacteriohopanepolyols. Organic Geochemistry 88, 1-16.

Imhoff, J.F., 2001. Transfer of *Rhodopseudomonas acidophila* to the new genus *Rhodoblastus* as *Rhodoblastus acidophilus* gen. nov., comb. nov. International Journal of Systematic and Evolutionary Microbiology 51, 1863-1866.

Jahnke, L.L., Summons, R.E., Hope, J.M., Des Marais, D.J., 1999. Carbon isotopic fractionation in lipids from methanotrophic bacteria II: the effects of physiology and environmental parameters on the biosynthesis and isotopic signatures of biomarkers. Geochimica et Cosmochimica Acta 63, 79–93.

Joyeux, C., Fouchard, S., Llopiz, P., Neunlist, S., 2004. Influence of the temperature and the growth phase on the hopanoids and fatty acids content of *Frateuria aurantia* (DSMZ 6220). FEMS Microbiology Ecology 47, 371–379.

Kannenberg, E.L., Poralla, K., 1999. Hopanoid biosynthesis and function. Naturwissenschaften 86, 168–176.

Karofeld, E., Toom, M., 1999. Mud-bottoms in Männikjärve Bog, Central Estonia. Proceedings of the Estonian Academy of Sciences, Biology and Ecology 48, 216-235.

Kelley, C.A., Dise, N.B., Martens, C.S., 1992. Temporal variations in the stable carbon isotopic composition of methane emitted from Minnesota peatlands. Global Biogeochemical Cycles 6, 263-269.

Kim, J.-J., Talbot, H.M., Zarzycka, B., Bauersach, T., Wagner, T., 2011. Occurrence and abundance of soil-specific bacterial membrane lipid markers in the Têt watershed (Southern France): Soil-specific BHPs and branched GDGTs. Geochemistry, Geophysics, Geosystems 12 DOI: 10.1029/2010GC003364.

Kip, N., van Winden, J.F., Pan, Y., Bodrossy, L., Reichart, G.-J., Smolders, A.J.P., Jetten, M.S.M., Sinninghe Damsté, J.S., Op den Camp, H.J.M., 2010. Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. Nature Geoscience 3, 617-621.

Kip, N., Dutilh, B.E., Pan, Y., Bodrossy, L., Neveling, K., Kwint, M.P., Jetten, M.S.M., Op den Camp, H.J.M., 2011. Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence of *Methylomonas* and *Methylocystis* in *Sphagnum* mosses from a Dutch peat bog. Environmental Microbiology Reports 6, 667-673.

Kip, N., Fritz, C., Langelaan, E.S., Pan, Y., Bodrossy, L., Pancotto, V., Jetten, M.S.M., Smolders, A.J.P., Op den Camp, H.J.M., 2012. Methanotrophic activity and diversity in different *Sphagnum magellanicum* dominated habitats in the southernmost peat bogs of Patagonia. Biogeosciences 9, 47-55.

Knani, M., Corpe, W.A., Rohmer, M., 1994. Bacterial hopanoids from the pink-pigmented facultative methylotrophs (PPFMs) and from green plant surfaces. Microbiology 140, 2755–2759.

Kool, D.M., Talbot, H.M., Rush, D., Ettwig, K., Sinninghe Damsté, J.S., 2014. Rare bacteriohopanepolyols as markers for an autotrophic, intra-aerobic methanotroph. Geochimica et Cosmochimica Acta 136, 114-125.

Kotsyurbenko, O.R., Friedrich, M.W., Simankova, M.V., Nozhevnikova, A.N., Golyshin, P.N., Timmis, K.N., Conrad, R., 2007. Shift from acetoclastic to $H_2$-dependent methanogenesis in a

West Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* Strain. Applied and Environmental Microbiology 73, 2344-2348.

Krivograd Klemenčič, A., Vrhovšek, D., 2003. Algae in the peat bogs Loverenška Jezera and Šijec in Slovenia. Natura Croatica 12, 141-150.

Krivograd Klemenčič, A., Smolar-Žvanut, Istenic, D., Griessler-Bulc, T., 2010. Algal community patterns in Slovenian bogs along environmental gradients. Biologia 65, 422-437.

Kuchta, T., Kubinec, R., Farkas, P., 1998. Analysis of hopanoids in bacteria involved in food technology and food contamination. FEMS Microbiology Letters 159, 221-225.

Kulichevskaya, I.S., Guzev, V.S., Gorlenko, V.M., Liesack, W., Dedysh, S.V., 2006. *Rhodoblastus sphagnicola* sp. nov., a novel acidophilic purple non-sulfur bacterium from *Sphagnum* peat bog. International Journal of Systematic and Evolutionary Biology 56, 1397-1402.

Kulkarni, G., Wu, C.-H., Newman, D.K., 2013. The general stress response factor EcfG regulates expression of the C-2 hopanoid methylase *hpnP* in *Rhodopseudomonas palustris* TIE-1. Journal of Bacteriology 195, 2490-2498.

Larmola, T., Tuittila, E.-S., Thijrola, M., Nykänen, H., Martikainen, P.J., Yrjälä, K., Tuomivirta, T., Fritze, H., 2010. The role of *Sphagnum* mosses in the methane cycling of a boreal mire. Ecology 91, 2356–2365.

Larmola, T., Leppänen, S.M., Tuittila, E.-S., Aarva, M., Merilä, P., Fritze, H., Tiirola, M., 2014. Methanotrophy induces nitrogen fixation during peatland development. Proceedings of the National Accademy of Science. USA 111, 734-739.

McClymont, E.L., Mauquoy, D., Yeloff, D., Broekens, P., van Geel, B., Charman, D.J., Pancost, R.D., Chambers, F.M., Evershed, R.P., 2008. The disappearance of *Sphagnum imbricatum* from Butterburn Flow, UK. Holocene 18, 991-1002.

McClymont, E.L., Bingham, E.M., Nott, C.J., Chambers, F.M., Pancost, R.D., Evershed, R.P., 2011. Pyrolysis GC–MS as a rapid screening tool for determination of peat-forming plant composition in cores from ombrotrophic peat. Organic Geochemistry 42, 1420-1435.

Metje, M., Frenzel, P., 2005. Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland. Applied and Environmental Microbiology 8191-8200.

Neunlist, S., Rohmer, M., 1985a. Novel hopanoids from the methylotrophic bacteria *Methylococcus capsulatus* and *Methylomonas methanica* (22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol and (22*S*)-35-amino-3β-methylbacteriohopane-30,31,32,33,34-pentol. Biochemical Journal 231, 635 - 639.

Neunlist, S., Rohmer, M., 1985b. The hopanoids of '*Methylosinus trichosporium*': aminobacteriohopanetriol and aminobacteriohopanetetrol. Journal of General Microbiology 131, 1363-1367.

Op den Camp, H.J.M., Islam, T., Stott, M.B., Harhangi, H.R., Hynes, A., Schouten, S., Jetten, M.S.M., Birkeland, N.K., Pol, A., Dunfield, P.F., 2009. Environmental, genomic and taxonomic

964 perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports 1, 293–
965 306.
966 Opelt, K., Berg, G., 2004. Diversity and antagonistic potential of bacteria associated with
967 bryophytes from nutrient-poor habitats of the Baltic Sea Coast. Applied and Environmental
968 Microbiology 70, 6569–6579.
969 Opelt, K., Chobot, V., Hadacek, F., Schönmann, S., Eberl, L., Berg, G., 2007. Investigations of the
970 structure and function of bacterial communities associated with *Sphagnum* mosses.
971 Environmental Microbiology 9, 2795–2809.
972 Ourisson, G., Rohmer, M., 1992. Hopanoids 2. Biohopanoids: A novel class of bacterial lipids.
973 Accounts of Chemical Research 25, 403-408.
974 Ourisson, G., Rohmer, M., Poralla, K., 1987. Prokaryotic hopanoids and other polyterpenoid sterol
975 surrogates. Annual Review of Microbiology 41, 301 - 333.
976 Pancost, R.D., Baas, M., van Geel, B., Sinninghe Damsté, J.S., 2000. $\delta^{13}C$ values and radiocarbon
977 dates of microbial biomarkers as tracers for carbon recycling in peat deposits. Geology 28,
978 663-666.
979 Pancost, R.D., Baas, M., van Geel, B., Sinninghe Damsté, J.S., 2003. Response of an
980 ombrotrophic bog to a regional climate event revealed by macrofossil, molecular and carbon
981 isotopic data. The Holocene 13, 921-932.
982 Pancost, R.D., McClymont, E.L., Bingham, E.M., Roberts, Z., Charman, D.J., Hornibrook, E.R.C.,
983 Blundell, A., Chambers, F.M., Lim, K.L.H., Evershed, R.P., 2011. Archaeol as a methanogen
984 biomarker in ombrotrophic bogs. Organic Geochemistry 42, 1279-1287.
985 Peiseler, B., Rohmer, M., 1992. Prokaryotic triterpenoids of the hopane series.
986 Bacteriohopanetetrols of new side-chain configuration from *Acetobacter* species. Journal of
987 Chemical Research-S 298-299.
988 Pearson, A., Flood Page, S.R., Jorgenson, T.L., Fischer, W.W., Higgins, M.B. 2007. Novel
989 hopanoid cyclases from the environment. Environmental Microbiology 9, 2175–2188.
990 Pearson, A., Leavitt, W.D., Sáenz, J.P., Summons, R.E., Tam, M.C.M., Close, H.G., 2009.
991 Diversity of hopanoids and squalene-hopene cyclases across a tropical land-sea gradient.
992 Environmental Microbiology 11, 1208–1223.
993 Pfennig, N., 1969. *Rhodopseudomonas acidophilla*, sp. n., a new species of the budding purple
994 nonsulfur bacteria. Journal of Bacteriology 99, 597-602.
995 Popp, T.J., Chanton, J.P., 1999. Methane stable isotope distribution at a Carex dominated fen in
996 north central Alberta. Global Biogeochemical Cycles13, 1063-1077.
997 Poralla, K., Härtner, T., Kannenberg, E., 1984. Effect of temperature and pH on the hopanoid
998 content of *Bacillus acidocaldarius*. FEMS Microbiology Letters 23, 253–256.
999 Poralla, K., Muth, G., Härtner, T., 2000. Hopanoids are formed during transition from substrate to
1000 aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiology Letters 189, 93-95.

1001 Quirk, M.M., Wardroper, A.M.K., Wheatley, R.E., Maxwell, J.R., 1984. Extended hopanoids in peat
1002 environments. Chemical Geology 42, 25-43.
1003 Raghoebarsing, A.A., Smolders, A.J.P., Schmid, M.C., Rijpstra, W.I.C., Wolters-Arts, M., Derksen,
1004 J., Jetten, M.S.M., Schouten, S., Sinninghe Damsté, J.S., Lamers, L.P.M., Roelofs, J.G.M., Op
1005 den Camp, H.J.M., Strous, M., 2005. Methanotrophic symbionts provide carbon for
1006 photosynthesis in peat bogs. Nature 436, 1153-1156.
1007 Rahman, M.T., Crombie, A., Chen, Y., Stralis-Pavese, N., Bodrossy, L., Meir, P., McNamara, N.P.,
1008 Murrell, J.C., 2011. Environmental distribution and abundance of the facultative methanotroph
1009 *Methylocella*. ISME Journal 5, 1061–1066.
1010 Rashby, S.E., Sessions, A.L., Summons, R.E., Newman, D.K., 2007. Biosynthesis of 2-
1011 methylbacteriohopanepolyols by an anoxygenic phototroph. Proceedings of the National
1012 Academy of Sciences USA 104, 15099-15104.
1013 Rethemeyer, J., Schubotz, F., Talbot, H.M., Cooke, M.P., Hinrichs, K.U., Mollenhauer, G., 2010.
1014 Distribution of polar membrane lipids in permafrost soils and sediments of a small high Arctic
1015 catchment. Organic Geochemistry 41, 1130-1145.
1016 Řezanka, T., Siristova, L., Melzoch, K., Sigler, K., 2011. *N*-acylated bacteriohopanehexol-
1017 mannosamides from the thermophilic bacterium *Alicyclobacillus acidoterrestris*. Lipids 46, 249–
1018 261.
1019 Ricci, J.N., Coleman, M.L., Welander, P.V., Sessions, A.L., Summons, R.E., Spear, J.R., Newman,
1020 D.K., 2014. Diverse capacity for 2-methylhopanoid production correlates with a specific
1021 ecological niche. ISME Journal 8,675–684.
1022 Ries-Kautt, M., Albrecht, P., 1989. Hopane-derived triterpenoids in soils. Chemical Geology 76,
1023 143-151.
1024 Rohmer, M., 1993. The biosynthesis of the triterpenoids of the hopane series in the Eubacteria: A
1025 mine of new enzyme reactions. Pure and Applied Chemistry 65, 1293 - 1298.
1026 Rohmer, M., Bouvier-Nave, P., Ourisson, G., 1984. Distribution of hopanoid triterpenes in
1027 prokaryotes. Journal of General Microbiology 130, 1137 - 1150.
1028 Rohmer, M., Ourisson, G., 1986. Unsaturated bacteriohopanepolyols from *Acetobacter aceti* ssp.
1029 *xylinum*. Journal of Chemical Research S, 356-357.
1030 Rosa-Putra, S., Nalin, R., Domenach, A.-M., Rohmer, M., 2001. Novel hopanoids from *Frankia*
1031 spp. and related soil bacteria. Squalene cyclization and significance of geological biomarkers
1032 revisited. European Journal of Biochemistry 268, 4300-4306.
1033 Rush, D., Sinninghe Damsté, J.S., Poulton, S.W., Thamdrup, B., Garside, A.L., Acuña González,
1034 J., Schouten, S., Jetten, M.S.M., Talbot, H.M., 2014. Anaerobic ammonium-oxidising bacteria:
1035 the only known biological source of the bacteriohopanetetrol stereoisomer in marine sediments.
1036 Geochimica et Cosmochimica Acta 140, 50-64.
1037 Sáenz, J.P., Sezgin, E., Schwille, P., Simons, K., 2012. Functional convergence of hopanoids and
1038 sterols in membrane ordering. Proceedings of the National Academy of Sciences of the United

States of America 109, 14236–14240.

Segers, R., 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41, 23-51.

Serkebaeva, Y.M., Kim, Y., Liesack, W., Dedysh, S.N., 2013. Pyrosequencing-based assessment of the bacteria diversity in surface and subsurface peat layers of a norther wetland, with focus on poorly studies phyla and candidate divissions. PLoS One 8, e63994. doi:10.1371/journal.pone.0063994

Shcherbakov, A.V., Bragina, A.V., Kuzmina, E.Y., Berg, C., Muntyan, A.N., Makarova, N.M., Malfanova, N.V., Cardinale, M., Berg, G., Chebotar, V.K., Tikhonovich, I.A., 2013. Endophytic bacteria of *Sphagnum* mosses as promising objects of agricultural microbiology. Microbiology 82, 306-315.

Simonin, P., Tindall, B., Rohmer, M., 1994. Structure elucidation and biosynthesis of 31-methylhopanoids from *Acetobacter europaeus*. European Journal of Biochemistry 225, 765-771.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Schouten, S., Fuerst, J.A., Jetten, M.S.M., Strous, M., 2004. The occurrence of hopanoids in Planctomycetes: implications for the sedimentary biomarker record. Organic Geochemistry 35, 561-566.

Spahni, R., Wania, R., Neef, L., van Weele, M., Pison, I., Bousquet, P., Frankenberg, C., Foster, P.N. Joos., F., Prentice, I.C., van Velthoven, P., 2011. Constraining global methane emissions and uptake by ecosystems. Biogeosciences 8, 1643-1665.

Spencer-Jones, C.L., 2016. Novel Concepts Derived from Microbial Biomarkers in the Congo System: Implications for Continental Methane Cycling (PhD thesis). Newcastle University, UK.

Spencer-Jones, C.L., Wagner, T., Dinga, B.J., Schefuβ, E., Mann, P.J., Poulsen, J.R., Spencer, R.G.M., Wabakanghanzi, J.N., Talbot, H.M., 2015. Bacteriohopanepolyols in tropical soils and sediments: sources and trends. Organic Geochemistry 89-90, 1-13.

Summons, R.E., Jahnke, L.L., Hope, J.M., Logan, G.A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400, 554-557.

Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., Asiegbu, F.O., 2014. Bacterial diversity and community structure along different peat soils in boreal forest. Applied Soil Ecology 74, 37-45.

Sundh, I., Mikkelä, Nilsson, M., Svensson, B.H., 1995. Potential aerobic methane oxidation in a *Sphagnum*-dominated peatland – controlling factors and relation to methane emission. Soil Biology and Biochemistry 27, 829-837.

Talbot, H.M., Farrimond, P., 2007. Bacterial populations recorded in diverse sedimentary biohopanoid distributions. Organic Geochemistry 38, 1212-1225.

Talbot, H.M., Watson, D.F., Murrell, J.C., Carter, J.F., Farrimond, P., 2001. Analysis of intact bacteriohopanepolyols from methanotrophic bacteria by reversed phase high performance

liquid chromatography - atmospheric pressure chemical ionisation - mass spectrometry. Journal of Chromatography A 921, 175-185.

Talbot, H.M., Squier, A.H., Keely, B.J., Farrimond, P., 2003a. Atmospheric pressure chemical ionisation reversed-phase liquid chromatography/ion trap mass spectrometry of intact bacteriohopanepolyols. Rapid Communications in Mass Spectrometry 17, 728-737.

Talbot, H.M., Summons, R., Jahnke, L., Farrimond, P., 2003b. Characteristic fragmentation of bacteriohopanepolyols during atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 17, 2788-2796.

Talbot, H.M., Rohmer, M., Farrimond, P., 2007a. Rapid structural elucidation of composite bacterial hopanoids by atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 21, 880-892.

Talbot, H.M., Rohmer, M., Farrimond, P., 2007b. Structural characterisation of unsaturated bacterial hopanoids by atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 21, 1613-1622.

Talbot, H.M., Summons, R.E., Jahnke, L.L., Cockell, C.S., Rohmer, M., Farrimond, P., 2008. Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. Organic Geochemistry 39, 232-263.

Talbot, H.M., Handley, L., Spencer-Jones, C.L., Dinga, B.J., Schefuß E., Mann, P.J., Poulsen, J.R., Spencer, R.G.M., Wabakanghanzi, J.N., Wagner, T., 2014. Variability in aerobic methane oxidation over the past 1.2 Myrs recorded in microbial biomarker signatures from Congo fan sediments. Geochimica et Cosmochimica Acta 133, 387-401.

Talbot, H.M., Bischof, J., Inglis, G.N., Collinson, M.E., Pancost, R.D., 2016. Polyfunctionalised bio- and geohopanoids in the Eocene Cobham Lignite. Organic Geochemistry. http://dx.doi.org/10.1016/j.orggeochem.2016.03.006

Taylor, K.A., Harvey, R.H., 2011. Bacterial hopanoids as tracers of organic carbon sources and processing across the western Arctic continental shelf. Organic Geochemistry 42, 487–497.

van Dongen, B.E., Talbot, H.M., Schouten, S., Pearson, N.P., Pancost, R.D., 2006. Well preserved Paleogene and Cretaceous biomarkers from the Kilwa area, Tanzania. Organic Geochemistry 37, 539-557.

van Dorselaer, A., Albrecht, P., Connan, J., 1975. Changes in composition of polycyclic alkanes by thermal maturation (Yallourn Lignite, Australia). In: R. Campos and J. Goni (Editors), Advances in Organic Geochemistry, 1975. Enadimsa, pp. 53-59.

van Winden, J.F., Talbot, H.M., Kip, N., Reichart, G.-J., Pol, A., McNamara, N.P., Jetten, M.S.M., Op den Camp, H.J.M., Sinninghe Damsté, J.S., 2012a. Bacteriohopanepolyol signatures as markers for methanotrophic bacteria in peat moss. Geochimica et Cosmochimica Acta 77, 52-61.

van Winden, J.F., Talbot, H.M., De Vleeschouwer, F., Reichart, G.-J., Sinninghe Damsté, J.S., 2012b. Variation in methanotroph-related proxies in peat deposits from Misten Bog, Hautes-Fagnes, Belgium. Organic Geochemistry 53, 73-79.

Vilcheze, C., Llopiz, P., Neunlist, S., Poralla, K., Rohmer, M., 1994. Prokaryotic triterpenoids: new hopanoids from the nitrogen-fixing bacteria *Azotobacter vinelandii*, *Beijerinckia indica* and *Beijerinckia mobilis*. Microbiology 140, 2749 – 2753.

Wagner, T., Kallweit, W., Talbot, H.M., Mollenhauer, G., Boom, A., Zabel, M., 2014. Microbial biomarkers support organic carbon transport from Amazon wetlands to the shelf and deep fan during recent and glacial climate conditions. Organic Geochemistry 67, 85-98.

Welander, P.V., Summons, R.E., 2012. Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. Proceedings of the National Accademy of Science of the USA 109, 12905–12910.

Welander, P.V., Hunter, R.C., Zhang, L., Sessions, A.L., Summons, R.E., Newman, D.K., 2009. Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas palustris* TIE-1. Journal of Bacteriology 191, 6145–6156.

Welander, P.V., Coleman, M.L., Sessions, A.L., Summons, R.E., Newman, D.K., 2010. Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proceedings of the National Accademy of Science of the USA. 107, 8537–8542.

Xie, S., Nott, C.J., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P., 2004. Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. Geochimica et Cosmochimica Acta 68, 2849-2862.

Xu, Y., Cooke, M.P., Talbot, H.M., Simpson, M.E., 2009. Bacteriohopanepolyol signatures of bacterial populations in Western Canadian soils. Organic Geochemistry 40, 79-86.

Yeloff, D., Bennett, K.D., Blaauw, M., Mauquoy, D., Sillasoo, U., van der Plicht, J., van Geel, B., 2006. High precision $^{14}C$ dating of Holocene peat deposits: a comparison of Bayesian calibration and wiggle-matching approaches. Quaternary Geochronology 1, 222–235.

Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W., Hunt, S.J., 2010. Global peatland dynamics since the Last Glacial Maximum. Geophysical Research Letters 37, L13402. http://dx.doi.org/10.1029/ 2010GL043584.

Zadorina. E.V., Slobodova. N.V., Boulygina. E.S., Kolganova. T.V., Kravchenko. I.K., Kuznetsov, B.B., 2009. Diversity of diazotrophic bacteria in peat soil by cloning of the *nifH* gene. Microbiology 78, 218-226.

Zhang, Z., Zimmermann, N.E., Kaplan, J.O., Poulter, B., 2016. Modeling spatiotemporal dynamics of global wetlands: comprehensive evaluation of a new sub-grid TOPMODEL parameterization and uncertainties. Biogeosciences 13, 1387-1408.

Zheng, Y., Singarayer, J.S., Cheng, P., Yu, X., Liu, Z., Valdes, P.J., Pancost, R.D., 2014. Holocene variations in peatland methane cycling associated with the Asian summer monsoon system. Nature Communications 5, DOI: 10.1038/ncomms5631

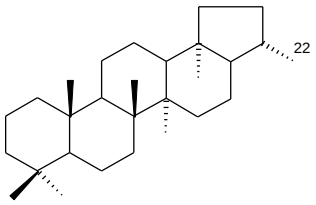
Zhu, C., Talbot, H.M., Wagner, T., Pan, J.M., Pancost, R.D., 2011. Distribution of hopanoids along a land to sea transect: Implications for microbial ecology and the use of hopanoids in environmental studies. Limnology and Oceanography 56, 1850–1865.

Zhu, B., van Dijk, G., Fritz, C., Smolders, A.J.P., Pol, A., Jetten, M.S.M., Ettwig, K.F., 2012. Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidising bacteria. Applied and Environmental Microbiology 78, 8657-8665.

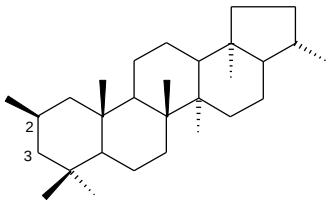
Zundel, M., Rohmer, M., 1985. Prokaryotic triterpenoids 3. The biosynthesis of 2β-methylhopanoids and 3β-methylhopanoids of *Methylobacterium organophilum* and *Acetobacter pasteurianus* ssp. *pasteurianus*. European Journal of Biochemistry 150, 35-39.

**Appendix 1.**

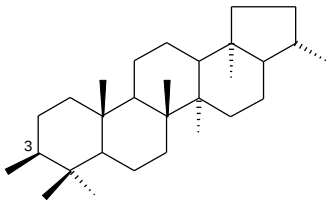
Ring system and side chains of BHPs observed in peat samples. Side chain structures h, i, n and o are based on LC-MS$^n$ analysis only. All other side chain structures shown have previously been unambiguously identified by NMR. When identified in this study using LC-MS only where stereochemistry can not be confirmed, we have assumed the structure to be the same as that previously characterised but the occurence of additional/alternative isomers of the side chain cannot be excluded.



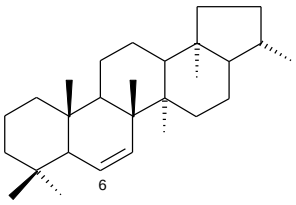

**I**



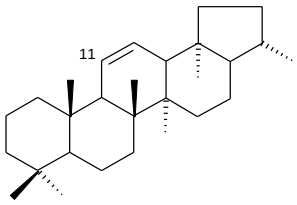

**II**



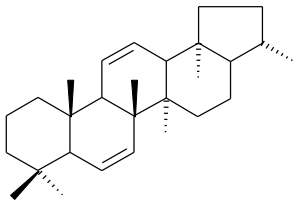

**III**



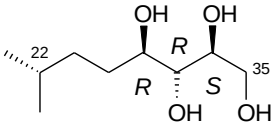

**IV**



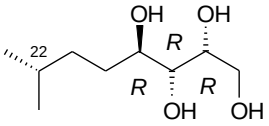

**V**



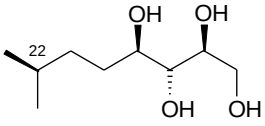
**VI**



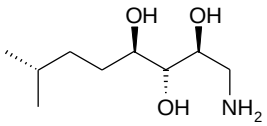

**a**



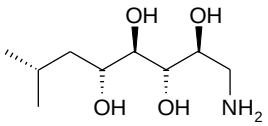

**b**



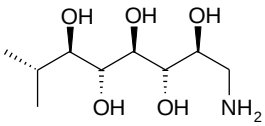

**c**



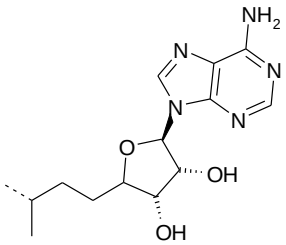

**d**



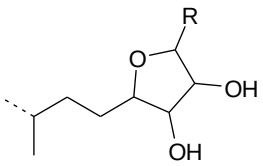

**e**



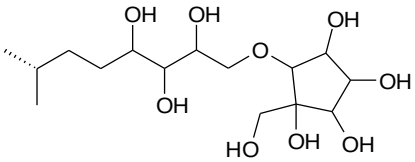

**f**



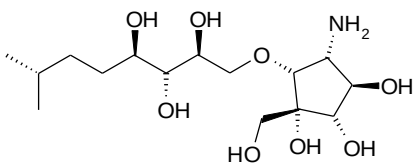

**g**



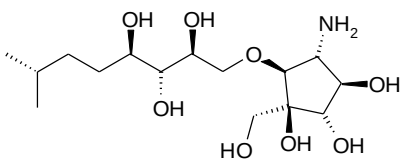

**h** (R = unknown)



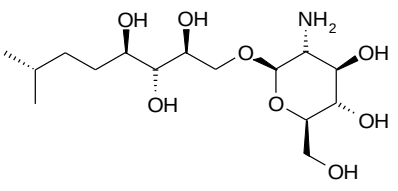

**i**



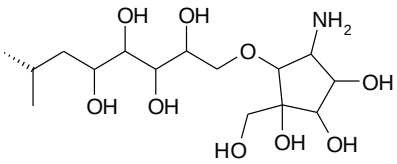

**j**



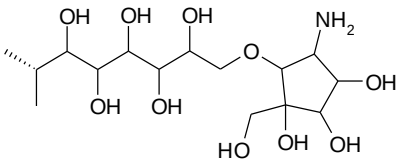

**k**



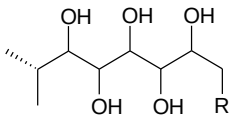

**m**




**n**




**o**




**p** (R= unknown)

1173

1174

1175

## List of Figures

**Figure 1.** Concentration (black bars; µg $g^{-1}_{TOC}$) and relative abundance (open diamonds) as % of total BHPs of dominant BHPs (a) BHT, (b) BHT-Cyclitol ether, (c) aminotriol, and methanotroph specific markers (d) aminotetrol and (e) aminopentol. Grey shaded area indicates region of water table variability (0-56 cm; Charman et al., 2007).

**Figure 2.** Concentration (black bars, µg $g^{-1}_{TOC}$) and relative abundance (open diamonds) as % of total BHPs of (a) combined unsaturated BHT [2 isomers], (b) unsaturated BHT-pentose, (c) BHT-pentose, (d) adenosylhopane (e) combined total 2-methyladenosylhopane, Adenosylhopane-group 2 and C-2 methylated adenosylhopane-group 2 (see appendix for individual structures). Grey shaded area indicates region of water table variability (0-56 cm; Charman et al., 2007).

**Figure 3.** Relative abundance of soil marker BHPs with depth in *Sphagnum* peat samples as % of total BHPs (a). Frequency of samples with relative abundance of soil marker BHPs in terrestrial samples in indicated ranges from (b) Peat, (c) Tropical and sub-tropical soils (d) Temperate soils and (e) Arctic samples (Key: OM and S = Organic matter and surface purmafrost soil; D = deep permafrost soils; IC = Ice complex; c = polygon centre; r = polygon rim). (Data from this study; Cooke et al., 2008a; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011; Zhu et al., 2011; van Winden et al., 2012a,b; Wagner et al., 2014; Doğrul Selver et al., 2015; Höfle et al., 2015; Spencer-Jones et al., 2015).

**Table 1.** Intact bacteriohopanepolyols identified in BM peat sample and potential sources known to be found in peat including heterotrophs, methanotrophs and phototrophs. (Note some species also produce other compounds not observed in this study; See Talbot et al., 2008 for review.)

| **APCI Base Peak ion (*m/z*)**<br>**BHP structure number**[b] | **746**[a]<br>**Ig** | **760**[a]<br>**IIg** | **761**<br>**Ih** | **775**<br>**IIh** | **712**<br>**IVd**<br>or<br>**Vd** | **714**<br>**Id** | **728**<br>**IId** | **728**<br>**IIId** | **772**<br>**Ie** | **830**<br>**If** | **653**<br>**IVa,b**<br>or **c** | **655**<br>**Ia** | **669**<br>**IIa** | **1002**<br>**Ij**<br>and/or<br>**Ik** | **1002**<br>**Im** | **1058**<br>**IVn**<br>**or**<br>**Vn** | **1060**<br>**In** | **1118**<br>**Io** | **1132**<br>**Ip** | **941**<br>**IVi**<br>**or**<br>**Vi** | **943**<br>**Ii** | **957**<br>**IIi** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HETEROTROPHS**[c] | | | | | | | | | | | | | | | | | | | | | | | |
| **PROTEOBACTERIA**<br>**Alphaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Acetobacter pasteurianus* (AAB)[d,e] | | | | | | | | | | | +[f] | + | | | | | | | | | | | Zundel and Rohmer, 1985 |
| *Acetobacter europaeus* (AAB) [d,e] | | | | | | | | | | | + | + | | | | | | | | | | | Simonin et al., 1994 |
| *Acetobacter aceti xylinus* (AAB) [d,e] | | | | | | | | | | | + | + | | + | + | + | + | | | | | | Talbot et al., 2007b |
| *Beijerinckia indica* | | | | | | + | | | | | | + | | | | | | | | | | | Vilcheze et al., 1994 |
| *Beijerinckia mobilis* | | | | | | + | | | | | | | | | | | | | | | | | Vilcheze et al., 1994 |
| *Bradyrhizobium* sp. | + | + | | | | + | | | | | | | | | | | | | | | | | Bravo et al., 2001 |
| *Methylobacterium spp.* (n = 8)[g,e] | | | | | | | | | | | | + | | + | + | | | | | | | | Knani et al., 1994 |
| *Zymomonas mobilis* | | | | | | | | | | | | + | | + | + | | | | | | | | Flesch and Rohmer, 1989 |
| **Betaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Burkholderia spp.* (n = 9) | | | | | | | | | | | | + | | + | + | | (+)[h] | | | | | | Cvejic et al., 2000b |
| **Gammaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Azotobacter vinelandii* | | | | | | | | | | | | | | + | | | + | | | | | | Vilcheze et al., 1994 |
| *Frateuria aurantia (AAB)* | | | | | | | | | | | | + | | + | + | | + | | | | | | Joyeux et al., 2004 |
| **Deltaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Geobacter spp.* (n = 2)[e] | | | | | | | | | | | | + | | + | + | | | | | | | | Eickhoff et al., 2013b |
| **Acidobacteria** | | | | | | | | | | | | + | | + | | | | | | | | | Garcia Costas et al., 2012 |
| **Actinobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Frankia spp.*[e] | | | | | | | | | | | | + | | | | | | | | | | | Rosa-Putra et al., 2001 |
| *Streptomyces coelicolor* A3(2) | | | | | | + | | | | | | | | | | | | | | | | | Poralla et al., 2000 |
| **Firmicutes** | | | | | | | | | | | | | | | | | | | | | | | |
| *Alicyclobacillus acidocaldarius*[e] | | | | | | | | | | | | + | | | + | | | | | | | | Poralla et al., 1984 |
| *Alicyclobacillus acidoterestris*[e] | | | | | | | | | | | | + | | | + | | + | + | | | | | Řezanka et al., 2011 |
| **Planctomycetes** | | | | | | | | | | | | (+) | | (+) | | | | | | | | | Sinnighe Damsté et al., 2004; Rush et al., 2014 |

## Table 1. Continued

| APCI Base Peak ion (*m/z*)<br>BHP structure number[b] | 746[a]<br>Ig | 760[a]<br>Iig | 761<br>Ih | 775<br>IIh | 712<br>IVd or Vd | 714<br>Id | 728<br>IId | 728<br>IIId | 772<br>Ie | 830<br>If | 653<br>IVa,b or c | 655<br>Ia | 669<br>IIa | 1002<br>Ij and/or Ik | 1002<br>Im | 1058<br>IVn or Vn | 1060<br>In | 1118<br>Io | 1132<br>Ip | 941<br>IVi or Vi | 943<br>Ii | 957<br>IIi | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| METHANOTROPHS | | | | | | | | | | | | | | | | | | | | | | | |
| **Alphaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Methylocella* spp. | + | | | | | + | | | | | | + | | | | | | | | | | | van Winden et al., 2012a |
| *Methylosinus* sp. | | | | | | + | | | + | | | + | | | | | | | | | | | Neunlist and Rohmer, 1985b |
| *Methylocystis* sp. | | | | | | + | | | + | | | | | | | | | | | | | | Talbot et al., 2001 |
| **Gammaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| *Methylovulum* sp.[e] | | | | | + | + | | | + | + | | | | | | | | | | | | | van Winden et al., 2012a |
| *Methylomonas* sp. | | | | | | | | | + | + | | | | | | | | | | | | | Neunlist and Rohmer, 1985a |
| *Methylococcus* sp.[e] | | | | | | | | | + | + | | | | | | | | | | | | | Neunlist and Rohmer, 1985a |
| *Methylocaldum* spp.[e] | | | | | | | | | (+) | + | | | | | | | | | | | | | Cvejic et al., 2000a |
| **Verucomicrobia** | | | | | | + | | | | | | | | | | | | | | | | | van Winden et al., 2012a |
| PHOTOTROPHS | | | | | | | | | | | | | | | | | | | | | | | |
| **Alphaproteobacteria** | | | | | | | | | | | | | | | | | | | | | | | |
| PNSB[i,e] | + | | (+) | | (+) | (+) | | | | | | (+) | (+) | (+) | (+) | | | | | | | | Talbot et al., 2007a |
| *Rhodoblastus sp.* | + | | | | | | | | | | | + | | + | + | | | | | | | | Talbot et al., 2007a |
| **Cyanobacteria[e]** | (+) | | | | | (+) | (+) | | | | (+) | (+) | (+) | (+) | (+) | | (+) | | | (+) | (+) | (+) | Talbot et al., 2008 |
| *Gloeocapsa sp.* | | | | | | | | | | | + | + | + | | | | | | | + | + | + | Talbot et al., 2008 |

[a] Di, tri- and tetraacetate forms are known and observed here.
[b] See Appendix for structures
[c] BHP producing organisms are listed by group (Heterotroph, Methanotroph, Phototroph) and within group by Phylum. Specific genera or species are listed only if considered to potentially be present in *Sphagnum* peat (see text for further details); alternatively only BHP biosynthesised by other members of the phylum/sub-group are indicated
[d] AAB = acetic acid bacteria
[e] indicates organism also produces other BHPs not identified in this study
[f] + = present in species (or all members of group tested to date; see Talbot et al., 2008 for review)
[g] number of species identified to produce BHPs
[h] (+) indicates compound found in some but not all tested members of group/genus

[i] PNSB = Purple non-sulphur bacteria.

Table 2. Total organic Carbon (%) and concentration of individual BHPs (μg $g^{-1}_{TOC}$) and grouped structural types in peat samples from Bissendorfer Moor.

| Base peak (m/z) | | 653 | 653 | 655 | 669 | 712 | 714 | 728 | 728 | 772 | 830 | 746 | 760 | 761 | 775 | 941 | 943 | 957 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth | | IVa, | IVa, | Ia | IIa | IVd or | Ib | IIb | IIIb | Ie | If | Ig | IIg | Ih | Ih | IVi or | Ii | IIi |
| cm | % TOC | b or c | b or c | | | Vd | | | | | | | | | | Vi | | |
| 0-2 | 41.440 | 21 | 18 | 20 | 6.4 | 0.00 | 69 | 1.4 | bdl[a] | 1.3 | bdl | 9.2 | bdl | bdl | 1.0 | 160 | 36 | bdl |
| 2-4 | 40.155 | 35 | 40 | 280 | 75 | 15 | 860 | 10 | bdl | 8.9 | bdl | 250 | 15 | 40 | 24 | 190 | 150 | bdl |
| 4-6 | 43.335 | 18 | 33 | 47 | 12 | 12 | 240 | bdl | bdl | bdl | bdl | 24 | bdl | 3.3 | bdl | 290 | 95 | bdl |
| 6-8 | 40.885 | 11 | 8.0 | 58 | 19 | 4.9 | 280 | bdl | bdl | 2.0 | bdl | 30 | bdl | 7.8 | 7.2 | 63 | 48 | bdl |
| 8-10 | 42.030 | 10 | 15 | 90 | 28 | 4.8 | 240 | 5.0 | bdl | 1.8 | bdl | 98 | 5.3 | 13 | 7.3 | 48 | 47 | bdl |
| 10-12 | 44.910 | 3.3 | bdl | 46 | 18 | 4.1 | 140 | bdl | bdl | 1.0 | bdl | 62 | bdl | 6.0 | 4.2 | 23 | 30 | bdl |
| 12-14 | 44.705 | bdl | bdl | 41 | 14 | bdl | 200 | bdl | bdl | 1.8 | bdl | 36 | bdl | 7.9 | 1.3 | 13 | 15 | bdl |
| 14-16 | 45.495 | 0.8 | bdl | 14 | 12 | 1.1 | 79 | 2.1 | bdl | 1.4 | bdl | 12 | 0.2 | bdl | bdl | 2.7 | 4 | bdl |
| 16-18 | 42.385 | bdl | bdl | 110 | 68 | bdl | 380 | 6.5 | bdl | 8.0 | bdl | 47 | bdl | bdl | bdl | 23 | 43 | bdl |
| 18-20 | 41.620 | bdl | 2.4 | 52 | 24 | 1.3 | 170 | 1.8 | 1.1 | 2.2 | bdl | 23 | bdl | bdl | bdl | 7.0 | 13 | bdl |
| 20-22 | 42.095 | bdl | bdl | 120 | 42 | bdl | 470 | 16 | 4.4 | 13 | bdl | 45 | 4.2 | bdl | bdl | 20 | 50 | bdl |
| 22-24 | 44.305 | bdl | bdl | 120 | 31 | 2.0 | 300 | 11 | 2.9 | 5.9 | 0.5 | 35 | 1.1 | bdl | bdl | 10 | 29 | bdl |
| 24-26 | 41.900 | bdl | bdl | 180 | 82 | bdl | 390 | 21 | 7.5 | 22 | 3.5 | 69 | bdl | bdl | bdl | bdl | 49 | bdl |
| 26-28 | 42.450 | bdl | bdl | 400 | 150 | bdl | 680 | 46 | bdl | 37 | 3.9 | 210 | bdl | bdl | bdl | bdl | 82 | bdl |
| 28-30 | 42.985 | bdl | bdl | 190 | 90 | bdl | 400 | 27 | bdl | 16 | 1.4 | 66 | 2.2 | bdl | bdl | bdl | 35 | bdl |
| 30-32 | 43.340 | bdl | bdl | 77 | 29 | bdl | 98 | 7.3 | bdl | 7.5 | bdl | 32 | 0.7 | bdl | bdl | bdl | 10 | bdl |
| 32-34 | 43.290 | bdl | bdl | 27 | 9.5 | bdl | 51 | 2.5 | bdl | 2.8 | bdl | 9.0 | 0.1 | bdl | bdl | bdl | 7 | bdl |
| 36-38 | 44.485 | bdl | bdl | 93 | 28 | bdl | 210 | 15 | 1.9 | 9.4 | bdl | 36 | bdl | bdl | bdl | bdl | 50 | bdl |
| 40-42 | 45.460 | bdl | bdl | 84 | 17 | bdl | 160 | 7.7 | bdl | 4.9 | 1.2 | 31 | 0.5 | bdl | bdl | bdl | 17 | bdl |
| 70-72 | 43.100 | bdl | bdl | 26 | 4.3 | bdl | 140 | 6.5 | bdl | 3.9 | 0.9 | 13 | bdl | bdl | bdl | bdl | 8 | bdl |
| 96-98 | 44.100 | bdl | bdl | 33 | 5.8 | 1.1 | 160 | 7.2 | 1.2 | 4.2 | 1.5 | 12 | 0.3 | bdl | bdl | 1.9 | 6 | bdl |
| 100-102 | 44.775 | bdl | bdl | 66 | 5.7 | bdl | 300 | 9.4 | bdl | 7.7 | 1.9 | 12 | bdl | bdl | bdl | bdl | 16 | bdl |
| 126-128 | 42.890 | bdl | bdl | 63 | 7.5 | 1.9 | 320 | 26 | bdl | 5.0 | 2.6 | 24 | bdl | bdl | bdl | 2.8 | 7 | bdl |
| 402-404 | 46.755 | 13 | 10 | 370 | 79 | 3.5 | 910 | 190 | 8.6 | 19 | 4.3 | 92 | bdl | 4.8 | bdl | 42 | 230 | 5.00 |
| 404-406 | 47.720 | 13 | 7.6 | 420 | 100 | 3.2 | 600 | 91 | 9.6 | 17 | 3.4 | 130 | 3.6 | 7.4 | bdl | 46 | 190 | 11.00 |
| 406-408 | 49.485 | 6.0 | 5.1 | 210 | 38 | 2.7 | 310 | 30 | 2.5 | 10 | 1.9 | 36 | bdl | 1.5 | bdl | 16 | 63 | 4.20 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 408-410 | 48.515 | 4.7 | 6.1 | 170 | 27 | 1.5 | 440 | 33 | 2.7 | 8.7 | 2.0 | 32 | bdl | 2.0 | bdl | 15 | 60 | 0.00 |

Table 2. Continued

| Base Peak (*m/z*) Depth cm | 1002 Ij and/ or Ik | 1002 Im | 1058 IVn or Vn | 1060 In | 1118 Io | 1132 Ip | Total μg $g^{-1}_{TOC}$ | % Tetra[b] | % Penta[b] | % Hexa[b] | % Soil[b] | % Unsat[b] | $R_{soil}$[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-2 | 50 | 3.2 | 3.9 | 8.9 | 14 | 15 | 438.3 | 87.8 | 3.2 | 6.6 | 2.3 | 46.3 | 0.34 |
| 2-4 | 560 | 28 | 4.8 | 45 | 30 | 44 | 2704.7 | 82.9 | 2.2 | 2.7 | 12.2 | 10.5 | 0.54 |
| 4-6 | 180 | 14 | 7.2 | 26 | 37 | 18 | 1056.5 | 89.1 | 3.1 | 5.2 | 2.6 | 34.1 | 0.37 |
| 6-8 | 110 | 12 | 1.2 | 6.0 | 12 | 4.2 | 684.3 | 89.7 | 1.3 | 2.4 | 6.6 | 12.9 | 0.44 |
| 8-10 | 110 | 9.0 | 1.9 | 11 | 12 | 4.3 | 761.4 | 79.7 | 1.9 | 2.1 | 16.2 | 10.5 | 0.58 |
| 10-12 | 100 | 7.7 | bdl | 4.3 | 3.6 | 3.7 | 456.9 | 81.4 | 1.2 | 1.6 | 15.8 | 6.7 | 0.61 |
| 12-14 | 91 | 2.1 | bdl | 6.6 | 4.8 | 1.4 | 435.9 | 86.3 | 1.9 | 1.4 | 10.4 | 3.0 | 0.52 |
| 14-16 | 34 | 0.9 | bdl | 1.2 | 0.7 | 0.0 | 165.9 | 90.7 | 1.6 | 0.4 | 7.4 | 2.8 | 0.47 |
| 16-18 | 180 | 8.7 | bdl | 7.4 | 7.9 | 2.5 | 892.0 | 91.8 | 1.7 | 1.2 | 5.3 | 2.6 | 0.30 |
| 18-20 | 69 | 2.6 | 0.2 | 2.2 | 2.9 | 0.6 | 375.3 | 91.7 | 1.2 | 0.9 | 6.1 | 2.9 | 0.31 |
| 20-22 | 400 | 18 | bdl | 13 | 5.4 | 2.3 | 1223.3 | 93.2 | 2.1 | 0.6 | 4.0 | 1.6 | 0.29 |
| 22-24 | 210 | 4.5 | bdl | 9.0 | 4.0 | 1.2 | 777.1 | 92.7 | 1.9 | 0.7 | 4.6 | 1.5 | 0.23 |
| 24-26 | 440 | 15 | bdl | 19 | 12 | 1.4 | 1311.4 | 90.3 | 3.1 | 1.3 | 5.3 | 0.0 | 0.28 |
| 26-28 | 930 | bdl | bdl | 28 | 8.3 | 5.0 | 2580.2 | 88.7 | 2.5 | 0.7 | 8.1 | 0.0 | 0.34 |
| 28-30 | 300 | 12 | bdl | 2.6 | 4.5 | 1.8 | 1148.5 | 91.8 | 1.6 | 0.7 | 5.9 | 0.0 | 0.26 |
| 30-32 | 65 | 2.0 | bdl | 1.7 | 0.9 | bdl | 331.0 | 87.1 | 2.8 | 0.3 | 9.9 | 0.0 | 0.30 |
| 32-34 | 45 | 1.2 | bdl | 0.7 | 0.4 | bdl | 155.8 | 91.7 | 2.2 | 0.3 | 5.8 | 0.0 | 0.25 |
| 36-38 | 180 | 7.6 | bdl | 4.3 | 2.5 | bdl | 637.7 | 91.8 | 2.1 | 0.4 | 5.6 | 0.0 | 0.28 |
| 40-42 | 110 | 1.5 | bdl | 1.9 | 1.2 | bdl | 437.9 | 90.7 | 1.6 | 0.5 | 7.2 | 0.0 | 0.27 |
| 70-72 | 150 | 2.5 | bdl | 2.1 | 0.9 | bdl | 358.4 | 94.2 | 1.7 | 0.5 | 3.6 | 0.0 | 0.33 |
| 96-98 | 130 | 2.0 | bdl | 1.0 | 1.3 | 0.5 | 369.3 | 94.4 | 1.4 | 0.9 | 3.3 | 0.8 | 0.27 |
| 100-102 | 260 | 3.3 | bdl | 3.3 | 2.3 | bdl | 687.6 | 96.0 | 1.6 | 0.6 | 1.7 | 0.0 | 0.15 |
| 126-128 | 400 | 5.8 | 0.2 | 5.0 | 2.5 | 1.0 | 874.2 | 95.4 | 1.2 | 0.7 | 2.7 | 0.6 | 0.28 |
| 402-404 | 740 | 25 | 0.7 | 23 | 8.3 | 4.6 | 2782.8 | 94.4 | 1.5 | 0.6 | 3.5 | 2.5 | 0.21 |
| 404-406 | 630 | 7.3 | 0.5 | 26 | 11 | 7.0 | 2334.6 | 91.2 | 1.9 | 0.9 | 6.0 | 3.0 | 0.25 |
| 406-408 | 340 | 3.7 | 0.3 | 9.0 | 7.7 | 2.9 | 1100.5 | 93.7 | 1.8 | 1.1 | 3.4 | 2.7 | 0.15 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 408-410 | 290 | 3.6 | 0.5 | 6.8 | 5.2 | 2.8 | 1113.6 | 94.6 | 1.4 | 0.9 | 3.1 | 2.5 | 0.17 |

[a] bdl = below detection limit;

[b] tetra = tetrafunctionalised BHPs with functional groups at C32, 33, 34 and 35, penta = pentafunctionalised with additional functional group at C31, hexa = hexafunctionalised with additional functional groups at C30 and 31, soil = all soil-marker BHPs inclidng adenosylhopane and related structures (**Ig**, **IIg**, **Ih**, **IIh),** unsat = the combined relative % of all unsaturated BHPs (tetra- and pentafunctionalised) with ring systems **IV** and/or **V**;

[c] $R_{soil}$ = (**Ig**+**IIg**+**Ih**+**IIh**)/(**Ia**+**Ig**+**IIg**+**Ih**+**IIh**) (Zhu et al., 2011).

1228

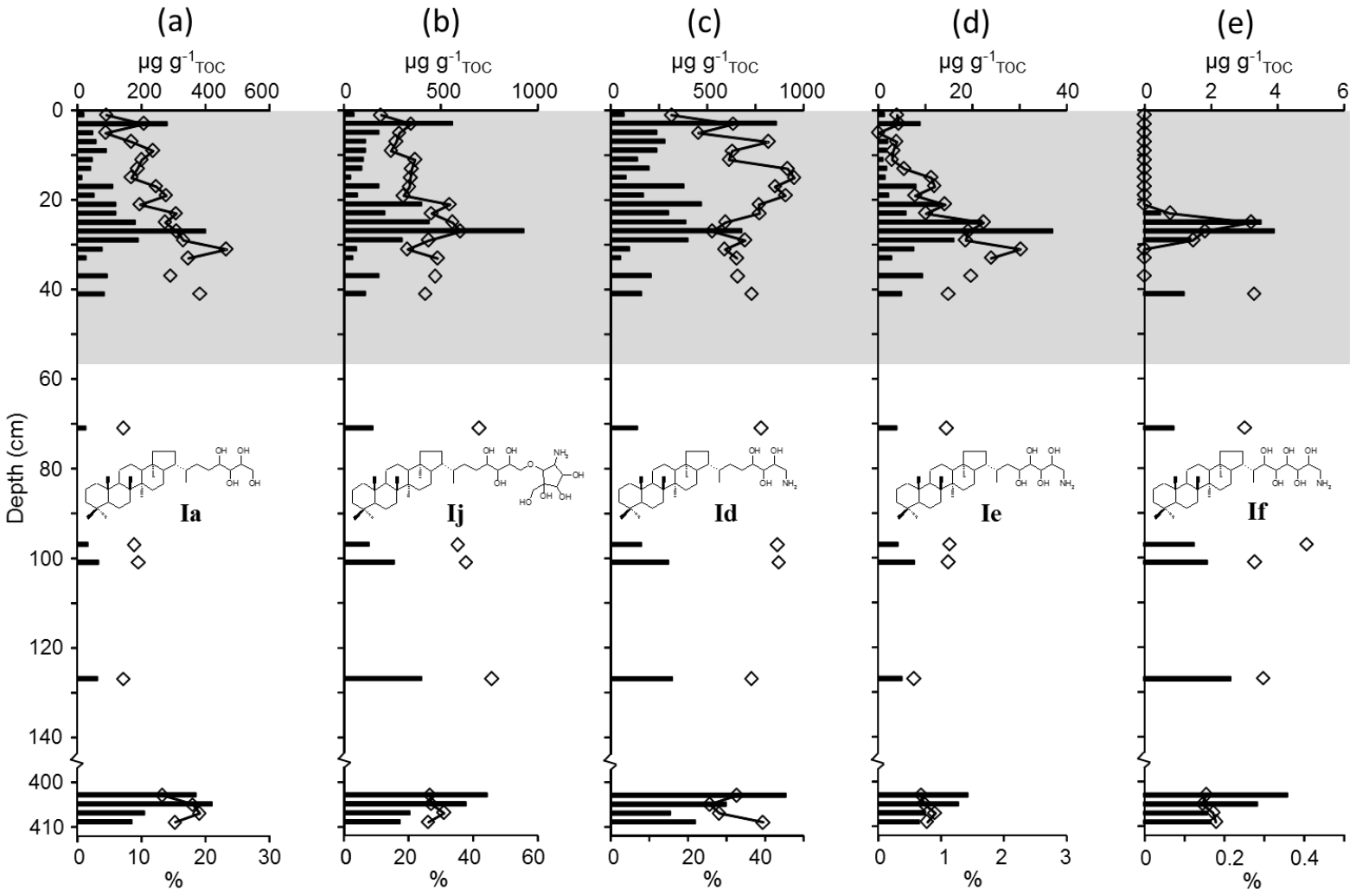



Figure 1

1229

1230

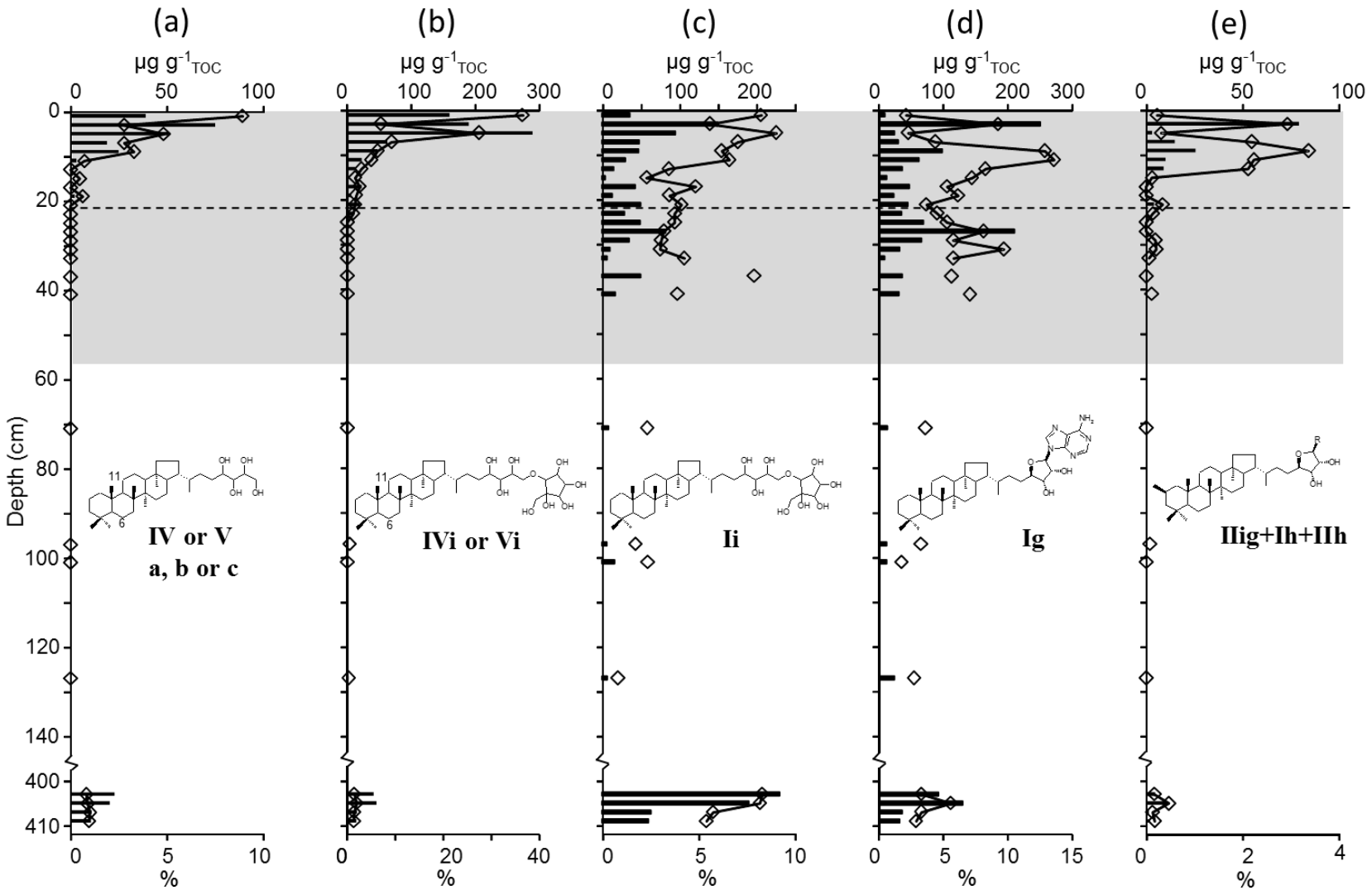



Figure 2

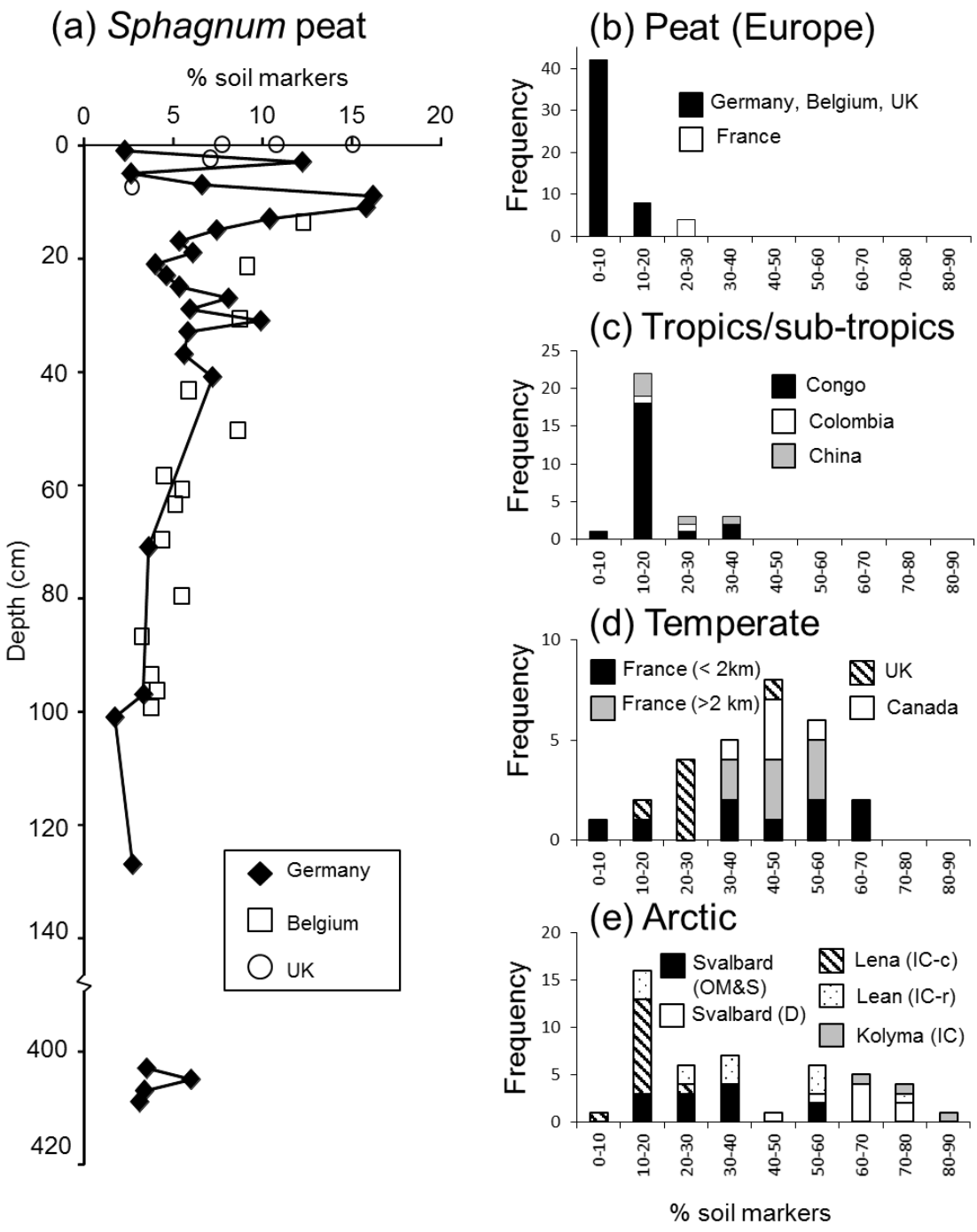



Figure 3

1233

1234